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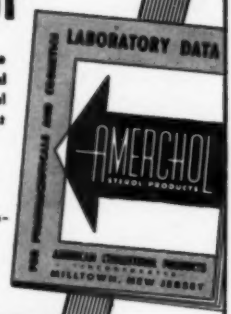

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VOLUME XL — *APRIL, 1951* — NUMBER 4 — CONSECUTIVE NO. 8

The Evaluation of "Vitamin P-Like" Compounds for Their Ability to Modify Capillary Permeability Using the Procaine-Bisulfite Method*,†

By THOMAS J. HALEY‡ and BONNIE M. RHODES‡, §

A large series of vitamin P-like compounds of varying basic structures has been evaluated for their capillary permeability effects by means of the mouse procaine-bisulfite method. It has been shown that: (*a*) the results obtained are independent of the *p*H value of the injected solutions, the solubilizers used, or the sugar(s) attached to the molecule; (*b*) that hydroxyl groups on the substituent benzene ring in positions 2 or 3 have played no part in potency under the experimental conditions employed; (*c*) that the glycoside linkage serves to detoxify and probably aid in solubilizing the flavonoid pigments; and (*d*) that the opening of the pyrone ring to form a chalcone derivative of phloroglucinol is essential to activity. The formation of a quinoid structure appears to be associated with physiological activity in the flavonoid series. However, phloroglucinol and the catechins are exceptions because quinones cannot be formed, yet the compounds have considerable activity. It has been suggested that the flavonoid compounds exert their capillary permeability effects by preventing relaxation of the precapillary sphincters of the capillary bed by inhibiting the conversion of ADP to ATP.

RECENT REPORTS, by Rekers and Field (6), that rutin decreased mortality in Roentgen-ray irradiated dogs, and by Griffith, *et al.* (2), that rutin hastened recovery in Roentgen-ray irradiated animals, resulted in a search for more potent "vitamin P-like" compounds.

This report will concern itself with the screening of a large series of vitamin P-like compounds for their ability to modify capillary permeability, and thus decrease the mortality due to intramuscularly injected procaine. An attempt will also be made to relate chemical structure to this physiological effect, and to point out the possible mechanism of action of the vitamin P-like compounds.

EXPERIMENTAL

Methods and Materials.—The procaine-bisulfite capillary permeability method of Richards and Kuetner (7) was adapted for use with mice instead of rats. Male albino mice weighing approximately 18 Gm. each were injected intravenously with varying doses of the vitamin P-like compounds (5–250

* Received August 29, 1950, from the University of California at Los Angeles.

† This paper is based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

‡ Division of Pharmacology and Toxicology, Atomic Energy Project, School of Medicine, University of California at Los Angeles.

§ The authors wish to thank the National Drug Co. for the esculin, 4-methylesculin, esculetin, and phosphorylated hesperidin; California Fruit Growers Exchange for the hesperidin, hesperidin methyl chalcone, and naringin; Hoffmann-La Roche for the epicatechin; Dr. James Couch of the Eastern Regional Laboratory of the Department of Agriculture for the rutin; and Abbott Laboratories for the methylglucamine use‘d in this investigation. The other compounds were purchased or synthesized in our laboratories.

mg./Kg.) ten minutes prior to the intramuscular injection of 500 mg./Kg. of aqueous procaine containing 0.1% of sodium bisulfite. Control animals received only the procaine-bisulfite injection after it had been observed that intravenous physiological saline had no effect on mortality. Both the control and the medicated groups were composed of ten animals each for each dosage level. The animals were injected in groups of five, alternating the medicated and the controls, to minimize the effect of slight fluctuations in room temperature on the overall results. The volume of vitamin P-like drug solution injected varied according to concentration, but was always less than 0.5 cc. The volume of the procaine-bisulfite solution injected rarely exceeded 0.11 cc. The end point employed was the prevention of death because, regardless of the dose of vitamin P-like compound, all of the animals showed typical procaine convulsions. The compounds were solubilized with methylglucamine according to the procedure of Richards and Kuetner (7). However, with calcium flavinate, esculetin, 4-methyl esculin, rhamnetin, iridin, hesperidin, homoeriodictyol, and rottlerin it was necessary to add from 0.1 to 0.8 cc. of 1 *N* NaOH per 10 cc. of solution, depending upon the concentration of the drug, to effect complete solution. The *p*H values for all the solutions, including those containing the NaOH, varied from 9.6 to 11.9 as measured with the Beckman *p*H meter. Control animals were injected with physi-

TABLE I.—EFFECTIVENESS OF FLAVONOIDS IN MODIFYING CAPILLARY PERMEABILITY

Drug	Chemical Name	Form	Molecular Weight	—Most Effective— Dose Mg./Kg.	mM/Kg.	Response, %
Rutin	3-Rhamnoglucoside of 3,5,7,3′,4′-pentahydroxyflavone	Glycoside	610	225	0.37	50
Quercitrin	3-Rhamnoside of 3,5,7,3′,4′-pentahydroxyflavone	Glycoside	448	50	0.11	43
Quercetin	3,5,7,3′,4′-Pentahydroxyflavone	Aglycone	302	25	0.08	37
Xanthorhamnin	3-Rhamnoside of 7-methoxy, 3,5,-7,3′,4′-pentahydroxyflavone	Glycoside	770	15	0.02	22
Rhamnetin	7-Methoxy-3,5,7,3′,4′-pentahydroxyflavone	Aglycone	316	50	0.16	29
Hesperidin	7-Rhamnoglycoside of 4′-methoxy-3,5,7,3′,4′-pentahydroxyflavone	Glycoside	610	25	0.04	57
Hesperidin methyl chalcone	7-Rhamnoglycoside of the methyl chalcone of 4′-methoxy-3,5,7,3′,-4′-pentahydroxyflavone	Glycoside	640–670	100	0.15 0.16	55
Phosphorylated hesperidin	Hesperidin containing sodium phosphate attached at 3′,7 and to the sugars	Glycoside	916	25	0.027	86
Hesperetin	4′-Methoxy-3,5,7,3′,4′-pentahydroxyflavone	Aglycone	302	Ineffective and toxic		
Calcium flavinate	Unknown	Unknown	Unknown	Ineffective and toxic		
Morin	3,5,7,2′,4′-pentahydroxyflavone	Aglycone	302	50	0.17	25
Iridin	7-Glycoside of 5,7,5′-trihydroxy-6,3′,4′-trimethoxyisoflavone	Glycoside	522	25	0.047	57
Irigenin	5,7,5′-Trihydroxy-6,3′,4′-trimethoxyisoflavone	Aglycone	360	Ineffective and toxic		
Irigenol	5,6,7,3′,4′,5-Hexahydroxyisoflavone	Aglycone	318	10	0.03	80
Homoeriodictyol	3′-Methoxy-5,7,3′,4′-tetrahydroxyflavanone	Aglycone	302	50	0.17	71
Naringin	5-Rhamnoglycoside of 5,7,4′-trihydroxyflavanone	Glycoside	580	75	0.13	80
Naringenin	5,7,4′-Trihydroxyflavanone	Aglycone	272	50	0.18	43
Phloridzin	2-Glycoside of the chalcone of 2,4,-6,4′-tetrahydroxydihydroflavanone	Glycoside	472	25	0.05	50
Phloretin	Chalcone of 2,4,6,4′-tetrahydroxydihydroflavanone	Aglycone	274	10	0.036	80
Maclurin	2,4,6,3′,4′-Pentahydroxybenzophenone	Aglycone	262	25	0.095	40
Phloroacetophenone	2,4,6-Trihydroxyacetophenone	Aglycone	168	15	0.089	80
Rottlerin	Contains phloroacetophenone group. Structure not completely known. ($C_{31}H_{32}O_8$ or $C_{30}H_{28}O_8$)	Aglycone	530 or 528	5	0.009	25
d-Catechin	3,5,7,3′,4′-Pentahydroxyflavan	Aglycone	290	50	0.17	71
dl-Epicatechin (33% *dl*-epicatechin)	3,5,7,3′,4′-Pentahydroxyflavan	Aglycone	290	25	0.09	62
Esculin	6-Glycoside of 6,7-dihydroxycoumarin	Glycoside	340	125	0.37	25
4-Methylesculin	6-Glycoside of 4-methyl-6,7-dihydroxycoumarin	Glycoside	353	Ineffective and toxic		
Esculetin	6,7-Dihydroxycoumarin	Aglycone	178	75	0.42	25
Phloroglucinol	1,3,5-Trihydroxybenzene	Aglycone	162	25	0.15	57

ological saline adjusted to *p*H 9.6, 10.0, 10.5, 11.0, 11.5, and 11.9, or with methylglucamine, glucose, or rhamnose at doses of 25 and 50 mg./Kg.

RESULTS

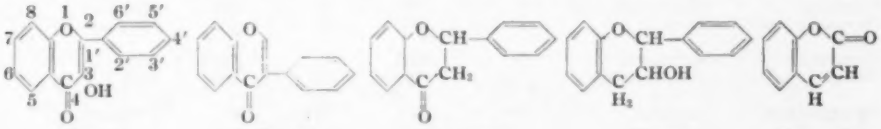
The injection of saline at various *p*H values, methylglucamine, glucose, or rhamnose had no effect upon the procaine-bisulfite mortality. The polyhydroxy phenolic compounds—ellagic acid, pyrogallol, resorcinol, rufigallol, quinalizarin, anthragallol, and naphthoresorcinol—were ineffective and toxic at doses from 5 to 150 mg./Kg. The results obtained with the various compounds are given in Table I, where the compounds are grouped according to their basic structures. Only the most effective doses are reported in order that a better comparison of potency can be obtained. Furthermore, all potency comparisons are on a mM/Kg. basis. The per cent response for each drug was calculated by the Sun-Shepard (9) formula to correct for medicated group mortality.

$$\text{Corrected per cent response} = \frac{100\ (\text{control mortality} - \text{medicated mortality})}{100 - \text{medicated mortality}}$$

Upon the basis of the data in Table I, the most potent compounds were: phosphorylated hesperidin irigenol, phloretin, hesperidin, iridin, phloridzin, phloroacetophenone, *dl*-epicatechin, maclurin, naringin, phloroglucinol, *d*-catechin, and homoeriodictyol, respectively. The other compounds showed potencies which were low and almost equivalent upon a mM/Kg. basis. It appears, although exceptions may be noted, that where a glycoside showed high activity at a low dose, its aglycone was ineffective or toxic within the range tested.

DISCUSSION

In general, the compounds tested belong to five general structural classifications: flavone, isoflavone, flavanone, flavan, and coumarin. Our discussion of the relationship between structural characteristics of these basic formulas will involve compounds such as phloretin, which are usually not included in the above groupings, but they can be included when one considers that in solution the vitamin P-like compounds exist as equilibrium mixtures of open and closed ring forms, with the former predominating in alkaline solution (10).



Effect of Phenolic Hydroxyls on Substituent Benzene Ring in Position 2 or 3.—Examination of Table II reveals that changes in the position of the phenolic hydroxyl groups on the substituent benzene ring from positions 3′,4′ to 2′,4′ has little or no effect on the potency of compounds containing the flavone nucleus. Blocking the hydroxyl in position 4′ by conversion to methoxyl increased the potency, whereas such blocking should decrease potency if it depended upon free hydroxyls in the 3′,4′-positions. This is also true with the isoflavone nucleus where the potency was high regardless of whether the 3′,4′ hydroxyls were free or blocked. Blocking the 3′-position with methoxyl or its removal entirely did decrease activity slightly when the parent nucleus was flavanone. However, one would expect a much greater loss or no activity if these hydroxyls were essential for physiological action. Furthermore, high activity was seen with the dihydroflavanone nucleus, which has a hydroxyl in the 4′-position only. The high activity associated with the 3′,4′-hydroxyl groups on the flavan nucleus may depend upon other factors, such as the spatial arrangement of the hydroxyl in the 3-position of the flavan nucleus. The decreased activity of catechin as compared to *dl*-epicatechin points to this possibility rather than to the presence of the hydroxyls in the 3′,4′-position, as these latter are the same in both compounds. Furthermore, when one considers the simpler compound, maclurin, which has only medium activity and yet has hydroxyl groups in the 3′,4′-positions, it seems that such groups are not essential to physiological activity. Further evidence of the nonessential nature of the hydroxylated benzene ring is the fact that phloroacetophenone, which has a methyl group substituted for the benzene ring, has high physiological activity.

Effect of Hydroxyl Groups on Parent Structure.—The presence of the glycoside linkage blocking the hydroxyl group has but a slight effect upon the activity of most of the compounds studied. It appears from the data in Tables I and III that the presence of the sugar(s) exerts a detoxifying influence on the molecule because the aglycones are generally much more toxic than the glycosides from which they are derived. It is also probable that the sugar(s) aids in solubilizing the drugs to some extent, and thus the flavonoid glycosides are similar to the cardiac glycosides in this respect (1). Conversion of the hydroxyl group in position 7 of the flavone nucleus or in position 6 of the isoflavone nucleus to methoxyl has little or no effect on potency. It appears that the spatial configuration of the hydroxyl group on the flavan nucleus has considerable influence on potency because *dl*-epicatechin is twice as active as catechin. None of the other compounds show any great increase in activity which can be correlated with the presence of a hydroxyl in position 3; in fact, two of the most potent compounds, iridin and irigenol, have that position blocked with the substituent benzene ring. Although a number of the highly active compounds have the group —CO—CH=CH—, this grouping does not appear to be essential to high potency because such a resonating structure is absent in phloroacetophenone, phloroglucinol, phloridzin, phloretin, the catechins, maclurin, and the flavanones. It appears that the carbonyl group (—CO—) attached to the phloro-

TABLE II.—EFFECT OF PHENOLIC HYDROXYLS ON BENZENE RING IN POSITIONS 2 OR 3

Parent Structure	Compound	Positions of Ring Substituents 2'	3'	4'	5'	Activity/mM
Flavone	Quercetin	H	OH	OH	H	Low
Flavone	Hesperidin	H	OH	OCH_3	H	High
Flavone	Morin	OH	H	OH	H	Low
Isoflavone	Iridin	H	OCH_3	OCH_3	OH	High
Isoflavone	Irigenol	H	OH	OH	OH	High
Flavanone	Homoeriodictyol	H	OCH_3	OH	H	Medium
Flavanone	Naringin	H	H	OH	H	Medium
Flavan	*dl*-Epicatechin	H	OH	OH	H	High
Benzophenone	Maclurin	H	OH	OH	H	Medium
Dihydroflavanone	Phloretin	H	H	OH	H	High

glucinol nucleus between two phenolic hydroxyl groups, and *para* to a third such group, confers high potency on the compound. However, the catechins are exceptions, and we have no explanation for their high potency. The size of the group attached to the carbonyl carbon atom can vary to a certain degree, but it seems that methyl is more active than phenyl (phloroacetophenone > maclurin) and less active than substituted benzyl or phenethyl (irigenol or hesperidin > phloroacetophenone). Further investigation of such structural variations may point out compounds having a greater activity/mM/Kg.

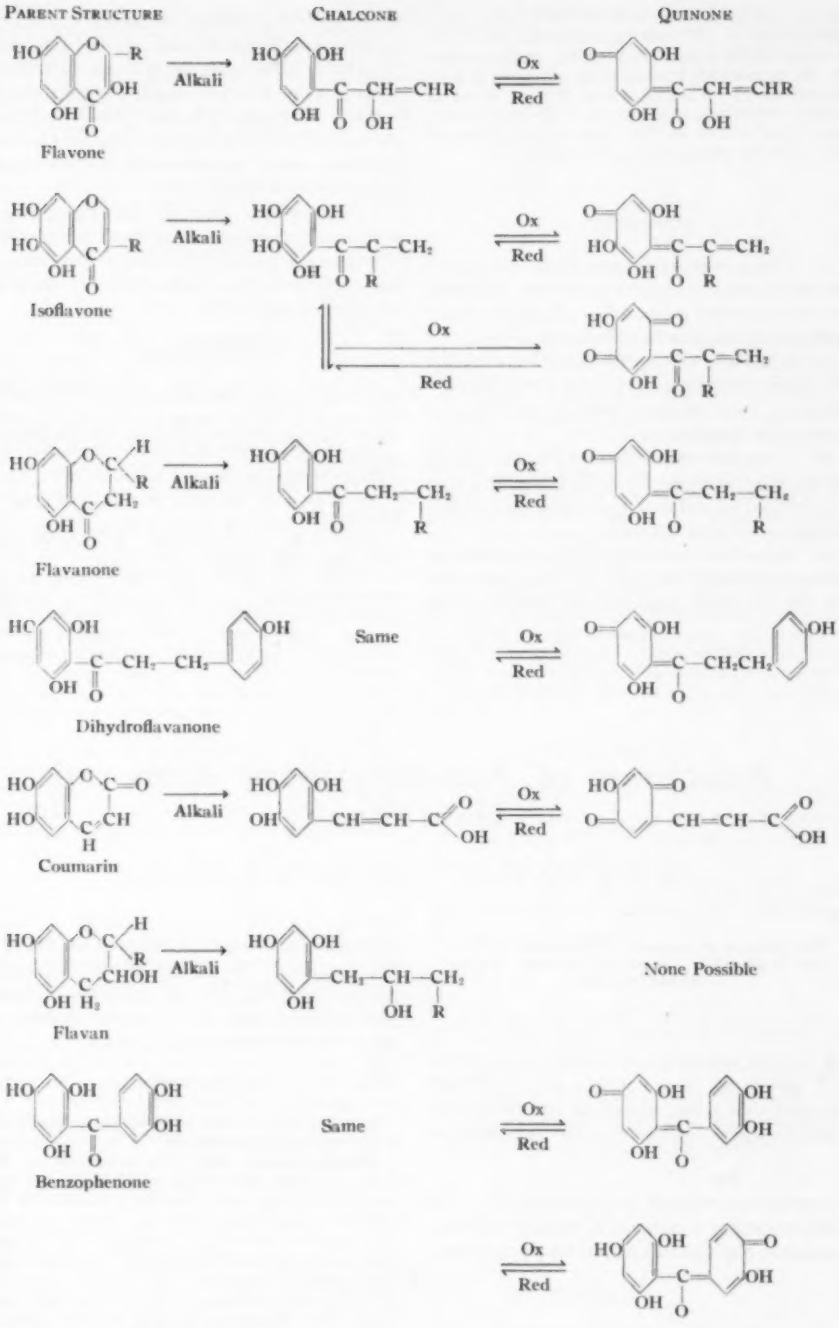
From the data in Tables I and III it is evident that all of the active compounds are phloroglucinol derivatives and that the phloroglucinol structure is essential for activity, since such phenolic compounds as ellagic acid, pyrogallol, resorcinol, quinalizarin, anthragallol, naphthoresorcinol, and rufigallol are entirely devoid of activity. When one considers the carbonyl group attached to the phloroglucinol nucleus, a quinoid structure becomes possible and may in no small way contribute to the high activity of compounds containing this system. The formulas on page 183 illustrate the quinone formation at equilibrium with the chalcone form.

Mode and Site of Peripheral Action.—Haley, *et al.* (3), demonstrated that various flavonoid compounds were potent vasoconstrictors when topically applied to the mammalian capillary bed. The site of action was the precapillary sphincters, the last muscular element on the arterial side of the capillary bed. Closure of these sphincters results in the preferential shunting of the blood flow into the larger vessels, by-passing the capillary bed and decreasing the interchange of materials between the circulating blood and the cells in the area. Thus, vasoconstriction with its resultant decreased filtration rate would indirectly influence capillary permeability and account for the prevention of death from procaine herein reported. The close relationship of the various compounds tested to phloridzin and the fact

TABLE III.—EFFECT OF HYDROXYL GROUPS ON THE PARENT FLAVONOID STRUCTURE

Parent Structure	Compound	Position of Ring Substituents 3	5	6	7	Activity/mM
Flavone glycoside	Rutin	O—S[a]	OH	H	OH	Low
Flavone	Quercetin	OH	OH	H	OH	Low, but greater than glycoside
Flavone glycoside	Xanthorhamnin	O—S	OH	H	OCH_3	Low, but greater than aglycone
Flavone	Rhamnetin	OH	OH	H	OCH_3	Low
Flavone glycoside	Hesperidin	OH	OH	H	O—S	High
Flavone	Hesperetin	OH	OH	H	OH	Toxic
Isoflavone glycoside	Iridin	H	OH	OCH_3	O—S	High
Isoflavone	Irigenin	H	OH	OCH_3	OH	Toxic
Isoflavone	Irigenol	H	OH	OH	OH	Higher than glycoside
Flavanone	Homoeriodictyol	H	OH	H	OH	Medium
Flavanone glycoside	Naringin	H	O—S	H	OH	Medium
Flavanone	Naringenin	H	OH	H	OH	Low
Flavan	*dl*-Epicatechin	OH (2)	OH (4)	H (6)	OH	High
Benzophenone	Maclurin	OH (2)	OH (4)	OH (6)	H	Medium
Dihydroflavanone glycoside	Phloridzin	O—S	OH	OH	H	High
Dihydroflavanone	Phloretin	OH	OH	OH	H	Higher than glycoside
Acetophenone derivative	Phloroacetophenone	OH	OH	OH	H	High
Coumarin glycoside	Esculin	H	H	O—S	OH	Low
Coumarin	Esculetin	H	H	OH	OH	Low

[a] S = Sugar (Glucose, rhamnose, or rhamnoglucose).



R = Substituted benzene ring.

that it inhibits the conversion of ADP (adenosinediphosphate) to ATP (adenosinetriphosphate) (4, 5, 8), essential for muscular relaxation, could account for the vasoconstriction necessary to prevent procaine fatalities. Thus, it appears that by acting as enzyme inhibitors, the vitamin P-like compounds exert their effects on the precapillary sphincters and indirectly on capillary permeability.

SUMMARY

1. A large series of vitamin P-like compounds of varying basic structures have been evaluated for their capillary permeability effects by means of the mouse procaine-bisulfite method.

2. It has been shown that the results obtained are independent of the *p*H value of the injected solutions, the solubilizers used, or the sugar(s) attached to the molecule.

3. It has been shown that hydroxyl groups on the substituent benzene ring in positions 2 or 3 have played no part in potency under the experimental conditions employed.

4. It has been shown that the glycoside linkage serves to detoxify and probably aid in solubilizing the flavonoid pigments. It also has been shown that the opening of the pyrone ring to form a chalcone derivative of phloroglucinol is essential to activity. The formation of a quinoid structure appears to be associated with physiological activity in the flavonoid series. However, phloroglucinol and the catechins are exceptions because quinones cannot be formed, yet the compounds have considerable activity.

5. It has been suggested that the flavonoid compounds exert their capillary permeability effects by preventing relaxation of the precapillary sphincters of the capillary bed by inhibiting the conversion of ADP to ATP.

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Association of Aurothiocarboxy Acids and Their Salts*

By E. E. MOORE, R. J. OHMAN† and B. MARCH

The stability of aurothio compounds in solution is shown to be related to their degree of association.

AQUEOUS SOLUTIONS of aurothio compounds are usually very unstable, but it has been reported that those of sodium aurothiomalate, $NaOOCCH_2CHCOONa$ (SAu), can be sterilized by Tyndallization without decomposition (1). The hitherto-unknown compound, sodium aurothioitamalate, $NaOOCCH_2CHCOONa$ (CH_2SAu), has now been synthesized. Because of the high alkalinity of this compound, the corresponding monosodium salt was prepared and its properties compared with those of monosodium aurothiomalate and other aurothio compounds.

* Received September 1, 1950, from the Research Division of Abbott Laboratories, North Chicago, Ill.
† Present address: University of Colorado, Boulder, Colo.

EXPERIMENTAL

Thioitamalic acid was prepared by the method of Holmberg and Schjanberg (2).

Aurothioitamalic Acid.—To a solution of 49.2 Gm. (0.30 mole) thioitamalic acid in 400 cc. of water was added slowly, with stirring, a solution of 30.4 Gm. (0.10 mole) of auric chloride in 100 cc. of water. The solution was filtered through a fritted glass filter, and evaporated to dryness on a steam bath under reduced pressure. The resulting jelly was triturated with several portions of ethyl acetate to remove the dithioitamalic acid formed. The resulting white powder was dried at 80° C. under vacuum. The yield was 34.2 Gm. (95% theory).

TABLE I.—PHYSICAL CONSTANTS OF 0.15 *M* SOLUTIONS

Compound	Freezing Point Depression	Specific Conductance	*p*H	Stability of Solution
Thiomalic acid	0.32	0.00328	2.05	
Aurothiomalic acid	0.03	0.00249	2.12	
Monosodium aurothiomalate	0.19	0.00746	5.16	*b*
Thioitamalic acid	0.29	0.00204	2.35	
Aurothioitamalic acid	0.02	0.00105	2.45	
Monosodium aurothioitamalate	0.17	0.00722	5.92	*a*
Sodium ω-aurothiopropionate	0.19	...	8.85	*c*
Sodium ω-aurothiobutyrate	0.16	...	...	*b*
Sodium ω-aurothiocaproate	0.15	...	...	*a*
Gold sodium thiosulfate	0.87	...	...	*d*

Anal.—Calcd. for $C_5H_7O_4SAu$: C, 16.67; H, 1.96; Au, 54.73. Found: C, 16.54; H, 1.96; Au, 55.03.

The disodium salt was found to be strongly alkaline. The nearly neutral monosodium salt was prepared by adding one equivalent of sodium hydroxide to a concentrated aqueous solution of the acid, and pouring the resulting solution into ethyl alcohol, from which the salt precipitated.

Monosodium aurothiomalate was prepared in a similar manner from its acid.

For the stability determinations, solutions containing the equivalent of 5% gold were sterilized by filtration and filled into ampuls. These ampuls were then subjected to different conditions and inspected for color change and solid matter. The results are expressed as follows: (*a*) no change in appearance after one hour's autoclaving at 121° C., followed by storage at 105° F. for six months; (*b*) darkening and precipitate formation under the above conditions but no change in appearance after Tyndallization followed by six months' storage; (*c*) some darkening and haze formation on Tyndallization, followed by increasing darkening during subsequent storage; (*d*) complete breakdown on Tyndallization, or on six months' storage without any sterilization (Table I).

DISCUSSION

The specific conductance and *p*H data show that aurothiomalic and aurothioitamalic acids and their salts are appreciably ionized in solution. In spite of the dissociation, their freezing-point depressions are much less than the calculated values for the undissociated compounds (3, 4). The molecular weights of aurothiomalic and aurothioitamalic acids, calculated from their formulas, are 346.3 and 360.4, respectively. Assuming no dissociation, the molecular weights, calculated from their freezing point depressions, are 5,134 and 3,290. Thus the molecular aggregate of aurothiomalic acid must contain in excess of 8, and that of aurothioitamalic acid more than 14, formula weights. This indicates that there is a relationship between stability and association. The sodium salts of aurothiomonocarboxy acids $AuS(CH_2)_xCOONa$ (5) were studied to determine whether a similar relationship existed in this series. The freezing-point depressions show that these compounds are associated in solution, and that the degree of association increases with the length of the carbon chain. The stabilities of solutions of these compounds were found to increase in the same order. In both series the degree of association increases with distance between the aurothio and carbonyl groups. This could explain the observation of Delange (6) that aurothioglycolic and its sodium salt are so unstable that they cannot be obtained in pure state. Gold sodium thiosulfate, which has a normal freezing-point depression, indicating no molecular association, is very unstable in solution.

SUMMARY

Gold thioitamalic acid and its monosodium salt were synthesized and the physical properties of their solutions compared with those of other aurothio compounds.

The stability of aurothio compounds in solution was shown to be proportional to their degree of molecular association.

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Standard Tolerances for Pharmaceutical Compounding. VI. Additional Data on Liquid Preparations*

By SAMUEL W. GOLDSTEIN†

Continued purchases of liquid preparations that are compounded by many pharmacists for a known state drug inspector have yielded additional samples for tolerance studies. Studies of solutions that were previously reported have been repeated with new samples, and series of other solutions have been utilized. The data obtained from all the selective series indicate that 1.73 standard deviations yield reasonable and equitable tolerances for assayable prescription-counter products. The reliability of the ingredient-weight basis of tolerance assignment is again demonstrated.

A SYSTEM for assignment of tolerance standards to liquid prescription-counter products was reported earlier (1). The system places any assayable medicinal ingredient in one of four weight groups for which standard tolerances have been determined. The weight groups and tolerances were developed from statistical data obtained from series of different solutions. In order to test the system and the proposed tolerances, the present studies were undertaken with additional series of compounded solutions.

EXPERIMENTAL

The manner of procurement of the solutions and the basis for "selection" of the utilized samples were described in earlier publications (1, 2). Analyses were based upon official assay procedures.

The data obtained from the new series of samples and pertinent data from the earlier studies are summarized in Table I. This shows the group placement of each solution according to the weight of the ingredient; the tolerance derived from 1.73 standard deviations; and the tolerance assigned from a scale with graduated increments of 2.5%. The data from the earlier studies of seven series of solutions containing stable ingredients formed the basis for the following tolerance-by-weight classification:

Group I.—Ingredient weight 5 Gm. or more. Tolerance ±10.0%.

Group II.—Ingredient weight 2.25 to 4.99 Gm. Tolerance ±12.5%.

Group III.—Ingredient weight 0.5 to 2.24 Gm. Tolerance ±15.0%.

Group IV.—Ingredient weight 0.49 Gm. or less. Tolerance ±17.5%.

TABLE I.—CLASSIFICATION AND TOLERANCES OF COMPOUNDED SOLUTIONS

No. of Selected Samples	Solution	Wt. of Ingredient Requested, Gm.	Group Placement[a]	Calcd. Tolerance,[b] ±%	Assigned Tolerance, ±%
77	Ammon. Chlor. 20%[c]	17.50	I	7.82	7.5
191	Pot. Iodide N. F.[c]	15.00	I	8.65	10.0
159	Pot. Iodide N. F.	15.00	I	9.22	10.0
95	Pot. Permang. 3%[c]	2.66	II	11.02	12.5
27	Pot. Permang. 3%	2.66	II	11.96	12.5
90	Boric Acid 3%[c]	2.66	II	11.70	12.5
41	Boric Acid 3%	2.66	II	13.49	12.5
94	Pot. Permang. 2.5%[c]	2.22	III	14.00	15.0
121	Boric Acid 3%	1.77	III	13.73	15.0
160	Pot. Permang. 2%	1.77	III	13.95	15.0
74	Neo-Silvol 2%	1.18	III	13.89	15.0
86	Protargol 1%	0.89	III	14.73	15.0
190	Silver Nitrate 1%[c]	0.59	III	15.65	15.0
103	Silver Nitrate 2%	0.59	III	14.80	15.0
69	Neo-Silvol 2%	0.59	III	14.79	15.0
62	Pot. Permang. 0.5%[c]	0.44	IV	16.63	17.5
75	Neo-Silvol 1%	0.30	IV	16.35	17.5
127	Silver Nitrate 1%	0.30	IV	16.43	17.5
48	Pot. Permang. 1%	0.30	IV	16.25	17.5
100	Protargol 0.5%	0.30	IV	16.30	17.5

[a] Ingredient-weight groups: I, 5 Gm. or more; II, 2.25 to 4.99 Gm.; III, 0.5 to 2.24 Gm.; IV, 0.49 Gm. or less.
[b] 1.73 Standard deviations converted to per cent.
[c] Series reported earlier.

The data from the three repeated series and the ten new series of solutions substantiate the original tolerance-by-weight classification. Determination of 1.73 standard deviations for each series of the ten new solutions has yielded tolerances which are similar to the tolerances that would have been as-

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† Formerly Pharmaceutical Chemist, Maryland State Health Department. Present address: American Pharmaceutical Association, Washington, D. C.

signed to these solutions by the ingredient-weight classification.

Four ingredient-weight groups are utilized for the convenient classification of almost all liquid prescription-counter products. If a system based on five groups were desirable, group I would include ingredient-weights of 17.5 Gm. or more, and would be assigned a tolerance of ±7.5%. The tolerances assigned to groups II, III, IV, and V, would then be ±10%, ±12.5%, ±15%, and ±17.5%, respectively.

The effect of altering the sample procurement procedure was studied. Samples were purchased in all types of drugstores by persons not known by the pharmacists in most cases, and the pharmacists were not informed that the solutions were to be tested. Application of the selective procedure, which omits any sample showing a deviation greater than ±30%, eliminated 108 of the 418 samples of 3% boric acid solution, 2 oz. (1.77 Gm. H_3BO_3) and 58 of the 444 samples of 2% Neo-Silvol solution, 1 oz. (0.59 Gm. Neo-Silvol). The determined tolerances represented by 1.73 standard deviations are ±20.0% for the boric acid solution and ±18.9% for the Neo-Silvol solution. These results would assign a tolerance of ±20% to both solutions. The recommended tolerance-by-weight system places these solutions in group III and assigns a ±15% tolerance. Two series of samples of these solutions were purchased by a known legal agent from pharmacists who were aware that their products would be tested. These samples yielded the data given in Table I. The different tolerances indicated for similar solutions by the two procurement procedures might be related to the extent of improvement attainable in compounding precision.

SUMMARY AND CONCLUSIONS

A major factor in the determination of reasonable and equitable tolerances for liquid prescription-counter products is the establishment of properly controlled conditions for experimental purposes. The tolerances obtained should be applicable to all assayable, extemporaneously compounded, liquid preparations. Experimental conditions have been utilized which yield reproducible results. The determined tolerance standards for stable ingredients appear to be reasonable and equitable, and their broad application has been substantiated. The tolerances assigned to solutions by the proposed system have been verified experimentally in every case that has been studied.

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An Ether Anesthesia Apparatus for Small Animals*

By THOMAS J. HALEY and R. W. DICKINSON†

SEVERAL METHODS have been used for the induction and maintenance of ether anesthesia in the rat and guinea pig. D'Amour and Blood (1) recommend the use of a Gooch crucible containing a cotton plug saturated with ether, Farris and Griffith (2) recommend the use of a wire and gauze mask, or the wrapping of the animal in a towel which is subsequently saturated with ether at the end enclosing the animal's head. However, all of these procedures suffer from the disadvantage of an inconstant depth of anesthesia due to evaporation of the anesthetic. Furthermore, there is the possibility of anesthetic death, due to too much ether becoming available when the anesthetic on the mask or cotton plug is renewed, as required from time to time. The disadvantages in these procedures are reflected in the length of time required for surgery, as well as in the loss of valuable animals. We would like to describe a simple apparatus, based on the apparatus used by Quigley and Heath (3), which not only overcomes the disadvantages of the older methods, but is also sufficiently automatic so that surgical procedures may be carried out on the rat and guinea pig with a minimum amount of attention to the anesthesia.

CONSTRUCTION OF APPARATUS

The chamber body is composed of a 2-in. length of $1^1/_2$ in. outside diameter by $^1/_8$-in.-thick-wall Lucite tubing, bored out at one end to $1^3/_8$ in. diameter for a length of $^3/_4$ in. A block of Lucite $^3/_4$ in. square and $^1/_2$ in. thick is shaped on one side to fit the outside of the Lucite tubing; it is then cemented to the

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† The authors wish to thank Dr. A. E. Guedel for his help during the designing of this apparatus.

tubing at the bored-out end in such position that approximately 1/4 in. of tubing is available for a cap to fit over the end. Through the center of this block a hole is drilled through to the chamber and tapped for 1/8 in. pipe to accommodate size No. 000 Lunkenheimer Lubricator (available at most hardware supply houses). A bronze wire screen is cut to 1 3/4 in. diameter and inserted on the shoulder of the tube. The tube end is loosely packed with cotton, and another similar screen, 1 1/2 in. in diameter, is placed over the end of the tube to enclose the cotton. This screen is held in place with a snug-fitting cap machined from 1/4-in.-thick Lucite, and provided with a through hole approximately 1 1/4 in. in diameter. The chamber assembly (see Fig. 1) is mounted on a shallow wood block machined to fit the outside of the tube, and held in place by a 3/4-in.-wide thin metal strap. The strap is tightened sufficiently so that the tube may be moved to and fro, as required during normal use. Rotation is prevented by a small strip of Lucite cemented along the bottom of the cylinder; this strip registers in a groove cut along the block. The whole assembly is conveniently mounted at the middle of one edge of a white-enameled board 15 in. square and 1/2 in. thick.

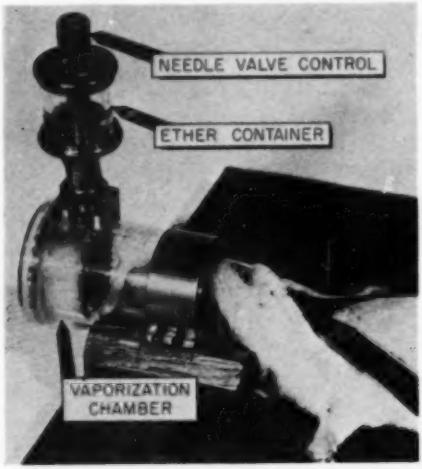

Fig. 1.—Use of etherizing apparatus.

USE OF APPARATUS

A rat or guinea pig is placed in a large, wide-mouthed jar having a tight-fitting lid and containing a cotton pad saturated with ether. When the animal has relaxed after the excitement stage of anesthesia induction, it is removed from the jar, taped to the operating board with cellophane tape, and its nose inserted into the ether chamber. The lubricator is filled with ether and regulated to the desired rate of delivery. Visual observation of the rate and depth of respiration, as well as the color of the blood in the vessels of the limbs, allows the surgeon to judge the depth of anesthesia and to increase or decrease the number of drops of ether in the observation tube of the lubricator by turning the thumb screw on the needle valve. The amount of air in the gas mixture can be regulated by moving the anesthesia chamber forward or backward as required. This apparatus can only be used with ether because Lucite is soluble in chloroform, other aliphatic chlorinated hydrocarbons, ketones, and aromatic hydrocarbons.

SUMMARY

An anesthesia apparatus, which can be built from readily available materials, has been described. This piece of equipment enables the investigator to maintain a relatively constant control over the depth of ether anesthesia in rats and guinea pigs, thus facilitating the completion of surgical preparation of such animals.

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Action of *Asarum canadense* and Its Combination with Senna*

By REBECCA C. HELLERMAN and MARIBELLE WOODS

Dealcoholized asarum fluidextract, when administered orally to mice, is relatively toxic in large doses, causing a depressant hypnosis followed by death. Addition of 1.0 per cent of the finely powdered asarum to the diet of mice for a period of eighty days did not affect food consumption or mortality. Studies on isolated mouse ileum indicate that asarum is capable of relaxing acetylcholine contractions, but when placed in the bath prior to acetylcholine it does not prevent a contraction. When placed in the lumen of the muscle it produces a long and continuous relaxation without injury to the muscle. If this exposure is sufficiently long it is capable of reducing the normal acetylcholine contraction. There is no indication that asarum *per se* is capable of causing catharsis. When very small doses are added to the standard cathartic dose of senna, there is an increase in cathartic activity, but with large doses the cathartic activity is decreased.

INTEREST in *Asarum canadense* stems largely from its empirical use in various pharmaceutical formulas, usually in combination with other botanical drugs. It was official in the seventh edition of the National Formulary (1) and Culbreth (2) describes it as a stimulant, carminative, tonic, diaphoretic, and diuretic. Studies were initiated to investigate in a preliminary manner its toxicological and pharmaceutical properties, since no experimental data are available in the literature. For the purposes of these investigations, emphasis has been placed on the possible role that asarum might play in modifying the cathartic action of senna. Emprical use in this connection was based on reduction of undesirable side effects accompanying catharsis. Experimentally, such an adjuvant action would be indicated by any of several characteristic actions. Among these might be synergism with the neurohormones involved in catharsis, thus sensitizing minimal evacuation stimuli. Or, since griping is a pain due to excessive stimulation, an antagonistic or depressant action on the intestinal musculature might reduce this symptom.

Preparations.—In the course of the experiments the following preparations were used:

a. Finely powdered drug.

b. The fluidextract, which was dealcoholized over a water bath to a syrupy consistency and made up to the desired concentration with distilled water.

c. The volatile oil of asarum, which was used to prepare a "water" by adsorbing onto an insoluble powder, mixing with water, and filtering in the usual manner of the pharmaceutical art.

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EXPERIMENTAL

Acute Toxicity—Mice.—In order to determine whether toxicity is a factor to be considered in evaluating cathartic action of asarum, preliminary tests were made using dealcoholized fluidextracts representing 0.5, 1.0, and 2.0 Gm. of the drug *per se*. In keeping with the established custom of cathartic assays, the volume per mouse was maintained at 0.5 cc. for oral administration. A summary of these tests is presented in Table I. Administration of extract equivalent to 500 or 1,000 mg. of asarum per mouse resulted in a period of depression, loss of righting reflex, and in some cases an almost comatose state. The depression caused by these large doses is not of a typical analgesic or anesthetic type, since peripheral stimuli elicit a response. The results showed a low mortality at doses of 500 mg. or less per mouse.

TABLE I.—ACUTE TOXICITY IN MICE FOLLOWING ORAL ADMINISTRATION OF DEALCOHOLIZED FLUIDEXTRACT OF ASARUM*

Drug Strength, Gm./Cc.	Asarum/ Mouse, Mg.	Sex	Results
0.5	250	Male	2/20
1.0	500	Female	3/13
2.0	1,000	Male	10/10

* The dose was 0.5 cc. per adult mouse. Results are expressed as number of animals dead/number of animals tested.

Chronic Toxicity.—To evaluate the chronic toxicity of asarum, male and female mice were placed on diets containing 1.0% powdered asarum, with appropriate control animals receiving untreated food. The results of this experiment are summarized in Table II. It is apparent that this level of feeding asarum (approximately 35 mg./mouse/day) did not influence significantly either food consumption or mortality, although there appears to be a retardation of growth in the female mice. No evidence of catharsis was observed.

Catharsis.—Miller and Alexander (3) successfully used the mouse laxative assay procedure of Geiger (4) as modified by Hazleton and Fortunato (5) and Grote and Woods (6) as a biologic assay of senna.

TABLE II.—SUMMARY OF DATA FROM EIGHTY-DAY FEEDING EXPERIMENTS IN WHICH MICE RECEIVED CONTROL DIET AND DIETS WITH 1.0% POWDERED ASARUM ADDED

	Sex	Days	No. of Mice Start	No. of Mice Finish	Av. Weight, Gm. Start	Av. Weight, Gm. Finish	Mouse Days	Total Food and Asarum Consumed, Gm.	Av. Food and Asarum/ Mouse/ Day, Gm.	Total Asarum, Gm.	Av. Asarum/ Mouse/ Day, Gm.	Total Asarum/ Mouse, Gm.
Control	Male	81	15	1	20.4	27.0	727	2,906	3.9	...	...	...
1% asarum	Male	81	30	6	20.5	28.3	2,141	7,810	3.6	78.1	0.036	2.92
Control	Female	79	15	6	18.0	25.6	1,070	4,048	3.7	...	...	...
1% asarum	Female	79	30	8	18.0	20.0	2,257	7,778	3.4	77.8	0.034	2.65

This method was used to study the effect of asarum on the cathartic action of senna. Mature female mice were fed a constant diet of Wayne Fox Blox (Allied Mills, Inc., Peoria, Ill.) and water *ad lib.* After a forty-five-minute observation period, those mice were selected for test which did not show signs of soft stools or diarrhea. The animals were not used more often than once a week.

Stock senna suspensions (1.0 Gm./20 cc.) were made daily with boiling distilled water, and diluted to provide the selected dose. Dealcoholized fluidextract of asarum was added to the senna suspension and diluted with distilled water to provide the selected dosage of each drug in a final volume of 0.5 cc. Doses of 0.5 cc. of the suspension or combination were given to the mice by stomach tube. The mice were then placed in individual wire cages elevated over paper and left overnight. Fecal stains on the paper from the mice showing laxative action were easily distinguished from urine strains by reading the stains on the underside of the paper. On the same day the standard senna was given to groups of 10 mice each, while the senna and asarum mixture was given to groups of about 20 mice. The test was run on three different days and at three levels for standard senna and asarum mixtures. The dilutions of laxative material were chosen so as to obtain well-spaced responses. The dosage required to produce laxation in 50% of the animals is called the LaD_{50}. The results are summarized in Table III. Addition of asarum to senna in quantities up to, and equal to, the senna increases the laxative activity of senna. However, very large additions of asarum to senna (10:1 or more) decrease the laxative activity.

Isolated Mouse Intestine.—The isolated ileum of the mouse was suspended in the conventional manner in a smooth muscle bath containing Locke's solution, maintained at a temperature of 37.5.° Under these conditions, 0.2 cc. of acetylcholine (0.1 mg./cc.) added to the bath caused a uniform, maximum, sustained, and reproducible contraction.

The characteristic effect of asarum added to the bath is demonstrated in Fig. 1. Figure 1 (*a*) shows

TABLE III.—EFFECT OF ASARUM ON THE CATHARTIC ACTION OF STANDARD SENNA IN MICE

Senna[a]				Senna and Asarum[b]					
Senna/ Mouse, Mg.	No. Pos./ No. Mice	Positive, %	LaD_{50}, Mg.	Senna/ Mouse, Mg.	Asarum/ Mouse, Mg.	No. Pos./ No. Mice	Positive, %	LaD_{50}, Mg.	Index, *a*/*b*
10.0	24/30	80		10.0	0.5	52/67	78		
6.31	20/30	67	5.5	6.31	0.5	39/65	60	4.7	1.17
3.98	8/30	27		3.98	0.5	20/45	44		
10.0	25/30	83		10.0	1.0	42/50	84		
6.31	17/30	57	5.6	6.31	1.0	48/65	74	3.9	1.44
3.98	9/30	30		3.98	1.0	26/53	49		
10.0	24/30	80		10.0	5.0	48/68	71		
6.31	20/30	67	5.5	6.31	5.0	44/65	68	4.6	1.20
3.98	8/30	27		3.98	5.0	18/45	40		
10.0	25/30	83		10.0	10.0	48/65	74		
6.31	17/30	57	5.6	6.31	10.0	43/65	66	4.3	1.30
3.98	9/30	30		3.98	10.0	24/55	44		
10.0	14/20	70		10.0	100.0	45/60	75		
6.31	10/20	50	6.0	6.31	100.0	20/55	36	7.2	0.83
3.98	7/20	35		3.98	100.0	5/55	9		
10.0	50/65	77		15.82	250.0	63/90	70		
6.31	32/55	58	5.2	12.62	250.0	54/90	60	10.9	0.48
3.98	19/50	38		10.0	250.0	31/73	42		
10.0	27/35	77							
6.31	15/30	50	5.6	...	250.0	0/44	0	...	Not laxative
3.98	11/30	37							

[a] Dose of senna/mouse suspended in 0.5 cc. distilled water.
[b] Dose of senna/mouse suspended in asarum extract of adjusted concentration.

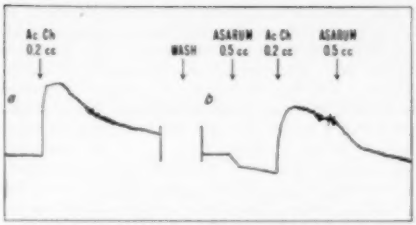

Fig. 1.—The effect of asarum (500 mg./cc.) on acetylcholine (0.1 mg./cc.) contractions of isolated mouse ileum.

a maximum acetylcholine contraction. If 0.5 cc. of dealcoholized fluidextract of asarum (500 mg./cc.) is added to the bath (Fig. 1 (*b*)), there is an immediate relaxation of the base line which does not prevent the subsequent contraction induced by acetylcholine. This contraction is gradually relaxed by the further addition of asarum to the bath.

To investigate the effect of asarum confined to the lumen of the isolated intestine, the method of Hazleton and Godfrey (7) was employed. After obtaining a control tracing and acetylcholine response (Fig. 2 (*a*)), the addition of 0.4 cc. of 250 mg./cc. dealcoholized fluidextract of asarum in the lumen at *b* resulted in a gradual but persistent relaxation of the test muscle, following the mechanically induced aberration in both tracings. The addition of asarum to the bath at *c* resulted in additional relaxation of the test muscle, but this was more pronounced in the control muscle. As shown in Fig. 2 (*d*), a dose of acetylcholine two and one-half times larger produced a proportional response in the control muscle, but only a minor contraction in the test muscle. In this tracing asarum was in the lumen of the test muscle and in the bath surrounding both muscles. The control muscle is therefore a reproduction of the conditions used in Fig. 1. As illustrated in Fig. 2 (*e*), washing of the lumen and the bath restores almost complete response to the larger dose of acetylcholine, and this response can again be relaxed completely by the addition of asarum to the bath.

In other experiments it was determined that during the early relaxant phase following the placing of asarum in the lumen there was no interference with

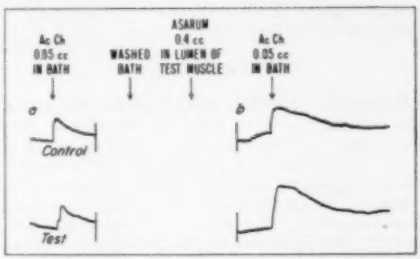

Fig. 3.—Synergistic action of asarum (10 mg./cc.) placed in the lumen of the test muscle with a minimum acetylcholine contraction (0.025 mg./cc.).

the acetylcholine contraction. The relaxation produced by the drug in the lumen is much more pronounced than that obtained from relatively large doses of asarum placed in the bath. Relaxation in some cases was so great that adjustment of the writing lever was necessary to compensate for the drop in the base line. Even after such extreme relaxation, however, washing of the lumen and the bath restored the response to acetylcholine.

When 0.5 cc. of 20 mg./cc. dealcoholized fluidextract of asarum is added to the bath, there is a very slight relaxation of a maximum acetylcholine contraction. Figure 3 (*a*) shows a minimum (0.05 cc. of 0.025 mg./cc.) acetylcholine contraction and Fig. 3 (*b*) shows that when 0.4 cc. of 10 mg./cc. asarum is placed in the lumen of the muscle, there is an enhancement of the minimum acetylcholine contraction. By comparison with the control muscle it will be noted that it is almost twice the previous contraction. If even this low concentration of asarum is allowed to remain in long contact with the lumen the stimulant action is lost and there is a gradual relaxation of the muscle similar to that obtained with the high concentrations.

To determine whether any or all of the activity of the fluidextract could be attributed to the volatile oil content, a "water" of asarum was prepared as described under "Preparations." The oil of asarum tends to have some of the same characteristics as the fluidextract, but in the concentration used is much less potent. When oil of asarum "water" is placed in the bath it tends to relax the acetylcholine contraction, but further relaxation can always

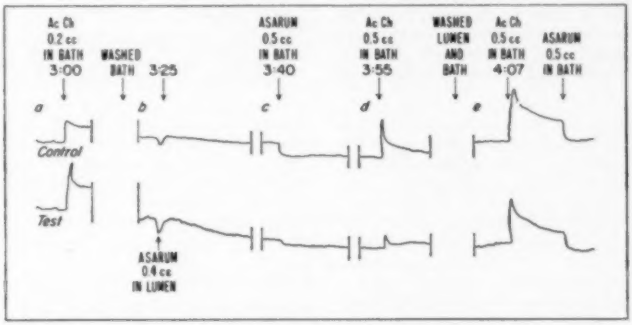

Fig. 2.—Consecutive tracings showing the effect of asarum (250 mg./cc.) on acetylcholine (0.1 mg./cc.) contractions of isolated mouse ileum.

be superimposed by the addition of the dealcoholized fluidextract of asarum.

DISCUSSION

Fluidextract of asarum is somewhat toxic following oral administration, in that 250 mg./mouse produces appreciable mortality and 1,000 mg./mouse is 100% lethal. In the higher dosage levels asarum produces a general depressant action comparable to hypnosis, not accompanied by analgesic action. Since no studies were directed at elucidating the systemic effects of asarum on the central nervous system, circulation, respiration, or peripheral muscle, it is not possible to define accurately the nature of this generalized depression.

The administration of 1.0% asarum in the diet of mice over a period of approximately eighty days does not appreciably affect the food consumption or mortality of the mice, but appears to retard the weight gain of the female.

Addition of asarum to senna in quantities up to, and equal to, the senna increases the laxative activity of senna. However, very large additions of asarum to senna (10:1 or more) decrease the laxative activity.

Studies on the isolated intestine of mice indicate that asarum added to the bath relaxes acetylcholine contractions, but that prior addition does not interfere with such contractions. When high concentrations are confined to the lumen of the intestine, however, asarum produced a prolonged and continuous relaxation which can be removed by washing. If exposure is sufficiently long, this is capable of reducing the normal acetylcholine contraction. When low concentrations of asarum are placed in the lumen there is enhancement of the acetylcholine contraction followed by relaxation of the muscle after a period of time.

Although direct comparison is not possible because of loss of some of the volatile oils in dealcoholizing the fluidextract, it does not appear that the oil of asarum is responsible for all of these observations.

While the present work gives no indication that asarum is capable of causing catharsis, it does show that asarum possesses pharmacological properties capable of modifying the cathartic action of other drugs. These properties seem to be consistent throughout the entire series of investigations. Experimentally, catharsis by senna results in expulsion of a soft, semifluid stool. In cathartic studies on mice, when asarum is added to the senna in concentrations up to the senna concentration, the cathartic action of the senna is slightly increased. This increase in activity with small doses is also illustrated in the smooth muscle lumen studies. However, if this is allowed to remain in prolonged contact with the lumen there is a relaxation which interferes slightly with the response of the muscle to acetylcholine. The fact that there is a slight synergism with small doses of asarum is probably insignificant, but it seems important that the intestinal musculature which is in a relaxed state due to the asarum is still capable of normal response to acetylcholine. In catharsis, acetylcholine is released following the release of irritant hydrolysis products from senna. The mechanism by which asarum produces relaxation without abolishing stimulation cannot be explained at this time.

CONCLUSIONS

1. Dealcoholized fluidextract of asarum is somewhat toxic to mice following oral administration of extract equivalent to 250–500 mg. of crude drug.

2. Administration of 1.0 per cent powdered asarum in the diet of mice over a period of eighty days is not toxic

3. Addition of asarum to senna up to 1:1 ratio has a slightly synergistic effect on catharsis; however, 10:1 doses have an antagonistic effect on catharsis.

4. Studies on isolated intestine show that 0.5 cc. of 500 mg./cc. asarum in the bath causes a relaxation which does not produce an immediate interference in the response to acetylcholine, but does reduce the response after a period of time.

5. Doses of 0.4 cc. of 10 mg./cc. asarum in the lumen synergize with acetylcholine, but when the asarum is allowed to remain in prolonged contact with the lumen there is a relaxation and eventually slight interference with acetylcholine contractions.

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Some Applications of Ion Exchange Resins to Medical Problems*,†

By EVAN W. McCHESNEY, FREDERICK C. NACHOD, and MAURICE L. TAINTER

A documented review of the present status of ion exchange resins as therapeutic agents is presented. The review indicates that anion exchange resins provide effective relief in the treatment of peptic ulcer. Certain cation exchange resins, used in conjunction with a limited salt intake, may be effective in the relief and control of edema. The resins that have been used as drugs are nontoxic at the dose levels required. It is predicted that additional therapeutic applications may be found for ion exchange resins.

IT IS NOT surprising that the ion exchange resins, which have proved so valuable in their applications to the problems of industry, of organic and inorganic chemistry, and of biochemistry, should have found their uses in medicine also. Since they are practically insoluble materials, they seem restricted to the oral route of administration.[1] Thus the resins should find their principal application in effecting an alteration of the pattern of ionic metabolism. Their use as physiological decontaminants was perhaps forecast many centuries ago by the employment of *terra sigillata* for the purpose of preventing the absorption of salts of heavy metals, especially $HgCl_2$. We may surmise that numerous medical applications will eventually be found, but thus far the important demonstrated uses are restricted to two. These were proposed in 1945 by Segal, Hodge, Watson, and Scott (1), and in 1946 by Dock (2). The former group proposed the use of a polyamine-formaldehyde resin (specifically, Amberlite IR-4) for the purpose of neutralizing gastric hyperacidity, while Dock proposed the use of cation exchange resins as a means of withdrawing or withholding sodium from the body. Both suggestions have been thoroughly examined in the laboratory and clinic, and both have proved feasible.

ANION EXCHANGE RESINS AS ANTACIDS

In their original paper, Segal and co-workers demonstrated that the weakly basic resins are capable of raising the *p*H of gastric juice from about *p*H 1 to 5, but found that discouragingly large quantities were required for the purpose. Furthermore, the quality of the result depended on the amount of agitation provided, as well as on the presence or absence of other materials, such as milk. These investigators fed the resin just as supplied, and did not investigate the possible benefits to be derived from a reduction of particle size. In 1946 Martin and Wilkinson (3) showed that 5 Gm. of Amberlite IR-4 had a neutralizing effect equal to that of 1 Gm. of dry $Al(OH)_3$ gel. When 600 cc. of fresh gastric juice, at *p*H 1.4, was stirred for a minute at 1,120 r. p. m. with 6 Gm. of Amberlite, the *p*H increased to 2. If the quantity of resin were doubled, the *p*H increased to 4.5. Speed of stirring was very important, and the difference in speed of action between 40-mesh and 200-mesh resin was almost infinite. The resin inactivated pepsin to the extent of 80% at *p*H 1, presumably by removal of HCl, but trypsin was reported to be irreversibly inactivated.

By 1947 several clinical trials of this resin had been reported. Kraemer and Lehman (4) described the treatment used successfully in 36 cases of Roentgenographically proved peptic ulcer. The dose was 3.5 Gm., given from four to eight times a day, as required by the patient. All cases were rendered pain-free in about a week, and remained so on prescribed diets plus resin. There were no toxic reactions, sensitivity, diarrhea, or constipation, but there were some complaints of throat irritation and objectionable taste. Spears and Pfeiffer (5) also treated a group of patients having ulcer pain. Only one of 30 failed to get relief from the administration of doses averaging 24 Gm./day, and in some cases the ulcer seemed to heal. The usual schedule of administration was 6 Gm. thirty minutes after each meal, and before bed time. There were some unpleasant side effects such as gagging, nausea, diarrhea, sense of fullness, dry stools, etc., but the over-all effect was favorable. One patient took 32 Gm. daily for a year without weight loss, detectable effect on blood count, permanent anacidity, or clinical avitaminosis. Kasdon (6) has successfully applied the same method, with less frequent medication, to the treatment of heartburn of pregnancy.

The advantages claimed for this type of treatment (5) as opposed to the numerous antacids which are effective by direct chemical reaction with HCl are the following: (*a*) great speed of action; (*b*) "greater neutralizing power when used in practical application"; (*c*) complete inhibition of peptic activity; (*d*) no removal of $PO_4^{=}$; (*e*) lack of acid rebound; (*f*) no constipating effect; and (*g*) no chloride removal.

More recently, Wirts and Rehfuss (7) have studied the effects of anion exchange resins on gastric and duodenal secretions and on gastric emptying time under somewhat different conditions from those

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† Presented at the Chicago meeting of the American Chemical Society, September 4, 1950.
[1] U. S. pat. 2,501,927 describes an anti-irritant powder, for external application, incorporating an anion exchange resin.

used by previous investigators. They felt that the most crucial test of an antacid would be that presented by an ulcer patient whose stomach is empty of food, but which contains a large volume of gastric juice of high acidity and peptic activity. These conditions were produced by histamine or insulin (0.1 u./Kg.) stimulation. The response of 20 ulcer patients to these stimuli was established, then the stimuli were repeated, giving antacids by stomach tube one hour later, and the effect on the titratable acidity and peptic activity of the gastric juice was observed. The antacid dosages chosen for study were 3.2 Gm. of Amberlite IR-4, 0.6 Gm. of $Al(OH)_3$ gel, or 0.6 Gm. of a modified Sippy powder (1 part $NaHCO_3$, 1 part $Bi_2O_3 \cdot H_2CO_3$, 2 parts $CaCO_3$). The Sippy powder was found to have the least effect on both pepsin and HCl concentrations, and also it invariably had only a transient neutralizing effect, leading to the familiar acid rebound. In some experiments the resin caused a more rapid decrease in gastric acidity than $Al(OH)_3$, and in others there was no essential difference. Similar conclusions were reached in nonulcer patients. When the antacids were given without histamine or insulin stimulation, the resin caused a more rapid decrease in gastric acidity than was obtained with $Al(OH)_3$ but after one hour's time the two effects could not be distinguished. They conclude that the anion exchange resin is effective in neutralizing acid and inactivating pepsin of both ulcer and nonulcer patients, but that its effects are not essentially different from those of other currently used methods of treatment. Further experimentation and experience will be required to tell whether the resin therapy has outstanding advantages. The present tendency seems to be to try to get along with suboptimal doses of resin, probably because of its rather high cost.

RELIEF AND CONTROL OF EDEMA

The theoretical background for the use of cation exchange resins for the purpose of sodium depletion is considerably more involved. Dropsy, or edema, is one of the oldest human afflictions, particularly of the aged. Dropsy patients appear to be waterlogged, and the obvious treatment is to restrict the intake of water. Actually, however, they are brinelogged; their ability to excrete sodium is impaired, and for this reason the rationale of restricting fluid intake becomes very doubtful. Although such prominent 18th century physicians as Thomas Witherly (8), George Baker, William Withering, and Francis Millman all advised free fluid intake in dropsy, restriction of fluid intake was very widely practiced[2] until the present century, when practically overwhelming evidence in favor of high fluid intake was presented (9–11).[3] It was in the fall of 1944 that the combination of low-salt, high-fluid intake was adopted at the Massachusetts General Hospital (10). Iseri, Boyle, Rosow, Griffin, and Engstrom (12) have recently reported that severe cardiac decompensation may be effectively controlled, and the edema diminished, simply by giving a synthetic liquid diet providing only 50 mg. of sodium per day.

Limitations of space will not permit us to consider all of the theoretical background involved in dropsy.[4] It must suffice to say that it develops as a result of some pathological process, the most common examples being congestive heart failure, the nephrotic stages of Bright's disease, cirrhosis of the liver, Cushing's syndrome, hypertension, etc. In all of these, the tendency of the patient to retain sodium is very great. There are, therefore, two methods of providing relief: (*a*) by restricting the salt intake one avoids the danger of further accumulations of fluid, and (*b*) by increasing the fluid intake one increases the volume of urine formed and thereby tends to wash out NaCl. As a means of restricting salt intake drastically, Kempner in 1944 (14) proposed a rice diet for the treatment of hypertensive patients. This consists of about 300 Gm. of rice, fruit, or fruit juice, and 100–500 Gm. of sugar daily. The diet provides about 350 mg. of NaCl, and is liberalized slightly only when the condition of the patient warrants it. Its gustatory limitations are obvious, but even so patients have subsisted on it for considerable periods of time. Of 500 cases treated, Kempner considered that 322 showed at least a reasonable improvement as regards blood pressure (15). Kert and co-workers (16) have shown ways by which a considerably more varied diet, yielding 2,400 cal., but only 200 mg. of Na daily, may be provided. This brings us now to the heart of the problem. It would be a very welcome thing indeed to these patients if they could consume a fairly normal diet containing 6–7 Gm. NaCl daily, which is ordinarily too much for them, and have half or more of the Na withheld from absorption. This is where the cationic exchange resins come in. However, to establish their use as an accepted adjunct form of treatment for edematous states, it was first necessary that both laboratory and clinical studies be done.

LABORATORY STUDIES

In his original paper, Dock (2) mentions feeding Ionac C200 and C284 to rats at a 10% level in the diet, and he found the sodium uptake in the intestine to be 20–30 mg./Gm. resin. No experimental details were given, but it was stated that calcium was also adsorbed. In 1949 Crismon (17) reported experiments involving feeding 10% of Dowex 50 (a nuclear sulfonic acid resin, in the H form) to rats. Sodium absorption was diminished markedly, and potassium absorption slightly. After nineteen days on this diet the blood levels of Na and K were normal, but Na, K, and Cl in the muscle were low. Thus far in 1950, two very detailed laboratory studies have appeared. Chen and Freeman (18) reported on the effects of feeding Duolite C-3 and Amberlite IR-100 in the H form, at a 10% level, to rats. Neither of these resins is of especially high capacity, but both produced marked effects on the absorption of minerals from the gastrointestinal tract. It was shown that the resins increase the fecal output of Na and K, but do not appreciably

[2] The usual supporting treatment consists of the administration of NH_4Cl, digitalis, and mercurial diuretics.

[3] And many others; for review of literature see Bridges, *et al.* (10).

[4] For a review of the literature on the mechanism of edem in cardiac decompensation, see Elkinton (13).

alter the absorption of Ca or P. On diets entirely devoid of Na and K and low in Ca there were large negative balances of Na, K, and Ca. It is of particular interest that the K deficits should be so much larger than those of Na, since one would suppose that these elements were being largely extracted from the digestive fluids, in which Na usually predominates over K by about 10 or 15 to one. The authors explain this on the basis that the resins have a higher affinity for K than for Na. As for combining capacity, they found that the resins bound about $^1/_3$ of their claimed (*in vitro*) capacities, and more Na was bound than K, except in the case of the Na- and K-free diet, where more K was bound than Na.

McChesney and McAuliff (19) also reported a study in rats using a variety of resins, dose levels, and dietary compositions. WIN 3000 (a sulfonic acid resin in the NH_4^+ form) was found to have an *in vivo* capacity of about 1 M. E./Gm., or 20% of its claimed *in vitro* capacity. It bound no Ca, so far as they could tell, and Na and K were bound in just about the ratios in which they were present in the diet. Amberlite XE-64 (a carboxylic resin, about the same in properties as IRC-50) had an *in vivo* capacity of about 2.5 M. E./Gm. or 25% of the claimed capacity (actually 40% of the capacity available at *p*H 7). It bound some Ca, and when fed partly in the form of the K resin it exchanged K for Na, thereby providing a means for removing Na from the food with less loss of fixed base than when fed in the H form. On a long-term experiment (twenty-one days) in which these two resins were compared at the 10% level and using a very low Na diet (0.05%), but with an adequate K content (0.48%), they were about equally effective in binding cations. Sodium and K were adsorbed in just about their dietary ratios, and positive balances of all of the elements were maintained. At the conclusion of the experiment, blood Na and K were essentially normal; muscle sodium was slightly low in some groups.

CLINICAL STUDIES

The number of clinical studies with cation exchange resins is thus far rather small, but investigations are going forward actively in a number of hospitals. The first studies were those reported at the 1948 Chicago meeting of the American Chemical Society by Greenblatt and Gilwood (20). They described treatment of 19 hospitalized patients with Permutit Z, a sulfonic acid resin in the ammonium form, the doses being 17 Gm., given thirty minutes before and after each meal. Of these patients, six responded positively; that is, by showing a definite and sustained increase in fecal Na. Five patients gave a partial response (the increase in fecal Na being of short duration) and the remaining eight subjects did not respond to the resin treatment, even when the dose was increased to 200 Gm. per day. At the Detroit Federation Meetings in 1949, Cobbey, Williams, MacRae, and Towery (21) reported studies involving one normal and one cardiac patient, using a resin of the carboxylic type. Fecal excretion of Na, K, Mg, and Ca were temporarily increased by feeding the resin, and there was an increase of urinary ammonia and titratable acidity. After four to eight days the resin seemed to lose its effect. No reason was suggested for this. However as noted above, similar failures have been recorded with other types of resins.

More recently Irwin, Berger, Rosenberg, and Jackenthal (22) presented a detailed and probably the best clinical study yet available, using Dowex 50H as the exchange resin. In a preliminary metabolic experiment, one noncardiac subject took the resin over a period of thirty-five days, in doses beginning at 25 Gm. and increasing step-wise to 60 Gm. The diet was constant, providing 6.7 Gm. of NaCl per day. The notable effects observed as a result of the resin feeding were a decrease in urinary output of Na and K, becoming more pronounced as the dose of resin increased. Fecal Na and K in general increased correspondingly, while Ca metabolism was essentially unaffected. Over the entire resin period the subject was in a state of positive Na, K, and Ca balance. However, his plasma CO_2-combining power fell by 30% of its original value, and the daily NH_3 production increased by as much as 750%. This indicated that he was in a state of compensated metabolic acidosis. (It should be kept in mind that for each 100 M. E. of cations removed there is an acidifying effect equal to that of ingesting 5.4 Gm. of NH_4Cl or 100 cc. of *N* HCl.) The accumulation of edema fluid in their regular cardiac patients could be prevented by doses of 50–60 Gm. resin per day, but it was necessary to watch them for symptoms of acidosis and K deficiency. The authors felt that the resin was being utilized to about $^1/_3$ of its claimed capacity in binding Na. This is even more favorable than some animal experiments indicate.

We have also been able to review a manuscript by Friedman and Greenblatt of Beth-El Hospital, Brooklyn (23), although it has not yet appeared in print. They have given detailed case reports on four individuals, typical of which is that of a male subject 68 years old having congestive heart failure and hypertension. In a preliminary four-day control period he excreted 0.2 Gm. of NaCl in the feces and 2.3 Gm. in the urine, although the diet provided 6.8 Gm. No digitalis, mercurials, or NH_4Cl were given during the period of observation. He then received 34 Gm. of Permutit Z three times daily for fourteen days. In the first four days the fecal Na (as NaCl) rose to 5.8 Gm. and the urinary Na (also as NaCl) to 3.5 Gm. After several days the excretion of Na by both routes diminished, whereupon they gave the resin in the form of enteric-coated capsules, with an apparent restoration of its original effect.[5] The patient lost 24 lb. during the fourteen days, which, if entirely derived from edema fluid, would represent a loss of about 92 Gm. of NaCl. The NaCl excreted was actually 67 Gm. above the subject's normal output, but the excess over intake was only 4 Gm., and this does not correlate with the weight loss. The patient's water intake was not controlled.

At the recent meeting of the Society for Clinical Investigation in Atlantic City, four papers dealing with the use of cation exchange resins in edema were presented, but so far they are available only in abstract form. These papers were given by Currens, Counihan, and Rourke (24), by Danowski and co-

[5] This apparent effect of enteric coating was entirely fortuitous, according to their present belief.

workers (25), by Kahn and Emerson (26), and by Kraus (27). Both carboxylic and sulfonic acid resins were employed, the latter usually in the NH_4 cycle. It seems to be generally agreed by these workers that resins of both types increase fecal loss of Na and K, but have little effect on fecal Ca. There is also an increase of serum Cl and urinary NH_4, and a compensated metabolic acidosis, as evidenced by a decrease of plasma CO_2-combining power of as much as 50%. The patients were almost invariably clinically improved, and promptly so, by either type of resin, the only untoward symptoms being occasional nausea, mild anorexia, and some constipation. It also seems to be generally agreed that what is most to be guarded against is K deficiency, although some mention is made of hypocalcemia and tetany. Kahn and Emerson make the interesting suggestion that cardiac patients might be fed a mixture of equal parts of carboxylic resin in the K and NH_4 cycles. They say that this combination will remove Na from the digestive fluids without loss of K. This would increase the intake of K so much, however, that it is hard to see how the efficiency of the resin, as regards Na removal, could fail to be considerably reduced.

In general, it would seem that patients have no difficulty in taking sufficient amounts of these resins to control or reduce their edema on a moderate salt intake, although higher available capacities would always be desirable. The required dose varies from one patient to another, but after some observation a maintenance dose for a moderate salt intake can be established. This is not a treatment to be undertaken lightly, or without adequate supervision. It seems to be agreed that the patient should be carefully watched for symptoms of renal damage, acidosis, low salt syndrome, and potassium deficiency. No evidence of thiamine deficiency has been observed in human subjects or experimental animals, but the theoretical danger exists, and probably occasional supplements should be given, perhaps at bed time, at least until more definite information on this point is available. Occasional calcium, and possibly magnesium and iron, supplementation might also be in order.

CHRONIC TOXICITY OF RESINS

Finally, reference should be made to the effects of long-term administration of ion exchange resins. Segal and co-workers (28) demonstrated that Amberlite IR-4 could be fed to rats at levels of 0.5, 2, and 50% for eight months without significant adverse effect on their growth or well-being, except that on the 20% resin regime the growth of male rats was somewhat inhibited. We (19) carried out a similar study with sulfonic acid resins at a 10% dietary level, in several different cycles, and found that the growth of the males, but not that of the females, was slightly inhibited by the acid-producing regimes. Since the males thrived on resin fed in the sodium cycle, the phenomenon seemed clearly related to acidosis, or perhaps to sodium-deprivation. There was no demonstrable pathology in any of the animals at autopsy after sixty days of resin feeding. There are several records (22, 23) of human subjects who have taken resins for long periods of time without serious adverse reaction. These and many other data[6] which are available constitute impressive evidence that it is possible to administer resins orally in the rather large doses which are required to alter the course of ionic metabolism.

SUMMARY

1. Numerous clinical reports indicate that anion exchange resins provide effective relief of X-ray proved peptic ulcer. Divided doses of about 25 Gm./day are usually recommended.

2. Cation exchange resins in either the H^+ or NH_4^+ form may be used for the relief and control of edema. Doses of about 50 Gm./day are usually required, in conjunction with a moderate salt intake. Since there are theoretical dangers involved in the treatment, the patients require close supervision.

3. The resins in both forms are relatively innocuous at the dose levels required.

4. There are probably other potential uses which have not received attention thus far.

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[6] See, for example, Heming, Flanagan, and Sax (29).

Considerations Involved in the Standardization of Polyvalent Respiratory Vaccines*

By F. J. MURRAY, KATHERINE A. LUDWIG, and MILTON J. FOTER

Certain aspects of standardization of polyvalent respiratory vaccines are discussed. A mouse-protection index may be used satisfactorily for the standardization of pneumococci or other mouse-virulent organisms. Of prime importance is the determination, under standardized conditions, that each lot is as good as those preceding.

THE STANDARDIZATION of biological products is generally highly desirable from the standpoint of the manufacturer, since it gives assurance that each lot is as good as those preceding. It is obvious that any standard selected should be one that could be achieved in the great majority of cases, and yet it should be rigid enough to be of real value. Consideration of the possibilities for standardizing polyvalent respiratory vaccines reveals a multiplicity of complications.

Since respiratory vaccines generally contain some organisms which are unable in the living state to infect animals, an animal-protection test is not possible for all of the constituents of such vaccines. The pneumococci, on the other hand, do lend themselves to a mouse-protection test so that a standard might be based on the antigenicity of these organisms. One must decide whether to test the individual suspensions of killed pneumococci before pooling, or the pooled material, or the finished product. Although an evaluation of the finished product would appear most meaningful, an evaluation of the individual vaccine suspensions is more practical and provides assurance that each lot is as antigenic as the last.

If the individual pneumococcus types contained in a given polyvalent vaccine are found to have the same antigenic activity, it is possible to establish one numerical index. If there is a variation between types, however, one must choose between using more than one index or using the lowest of the indexes. When the aim is merely to prove a lot "as good as" rather than to indicate "how good," this problem is of little importance.

We have previously described a method for comparing vaccines based on an LD_{50} calculation and expressed as a logarithm protection index × 10 (1). The logarithm of the LD_{50} of the control group was subtracted from the logarithm of the LD_{50} of each test group, the difference being multiplied by an arbitrary factor of 10. This method has been very satisfactory for comparing various lots of a respiratory vaccine[1] with regard to the individual pneumococcal types. In our studies, 18–20 Gm. albino mice were given a single intraperitoneal injection of 5×10^9 formalin-killed cells, and seven days later were challenged with a range of dilutions permitting calculation of the LD_{50} by the method of Reed and Muench (2).

Some idea of the type of range encountered and the reproducibility to be expected may be obtained from the data on various batches in Table I.

TABLE I.—ANTIGENICITY OF LOTS OF PNEUMOCOCCUS VACCINES

Vaccine, *D. pneumoniae*	Lot	No. Mice	LD_{50} Control	LD_{50} Lot	10 × Log Protection Index
I	A	48	$10^{-9.20}$	$10^{-3.00}$	62
	B	48	$10^{-9.20}$	$10^{-3.80}$	50
	C	48	$10^{-8.50}$	$10^{-3.02}$	53
	D	60	$10^{-8.60}$	$10^{-3.15}$	55
	E	48	$10^{-9.50}$	$10^{-2.37}$	71
	F	48	$10^{-9.60}$	$10^{-3.16}$	64
II	A	48	$10^{-9.00}$	$10^{-5.43}$	36
	B	48	$10^{-9.00}$	$10^{-5.50}$	35
III	A	48	$10^{-8.50}$	$10^{-5.43}$	31
	B	48	$10^{-8.62}$	$10^{-5.15}$	35
	C	48	$10^{-8.25}$	$10^{-4.53}$	37
	D	48	$10^{-9.00}$	$10^{-5.23}$	37
VII	A	48	$10^{-9.00}$	$10^{-6.00}$	30
	B	48	$10^{-9.00}$	$10^{-6.20}$	32
VIII	A	48	$10^{-9.24}$	$10^{-5.77}$	34
	B	48	$10^{-9.00}$	$>10^{-6.00}$	<30
	C	48	$10^{-8.75}$	$10^{-6.62}$	21

Obviously, types II, III, VII, and VIII could be expected to attain a protection index of 30 (protection against 1,000 LD_{50} doses) in every lot. Type I apparently is much more antigenic, and thus every lot of vaccine could be expected to reach an index of 50 (100,000 LD_{50} doses). In this case, if one index is desired, 30 would have to be the choice, but obviously 30 is much too lenient a requirement for our type I.

Our results have been uniform with mice from different sources of supply and with either sex.

* Received September 27, 1950, from the Department of Bacteriology, Research Laboratories, The Wm. S. Merrell Co., Cincinnati, Ohio.

[1] Oravax, registered trade mark of the Wm. S. Merrell Co.

Obviously, these factors should be checked and if it is found that a particular strain or sex gives an irregular or unusual response, this must be taken into consideration in the evaluation of results. The variables of animal tests are numerous, but fortunately more and more of them are being revealed. Results may be influenced by varying the dose of vaccine, or the waiting period before challenge, or even by varying the species of test animal. By the proper combination of factors a wide range of results may be obtained and this should tend to minimize the importance of a high protection index. It appears far more important to establish that under a standardized set of conditions one can repeatedly protect against a given number of challenging doses, rather than to strive for protection against higher and higher doses with the identical vaccine.

SUMMARY

1. Some aspects of standardization of polyvalent respiratory vaccines have been considered in this paper.

2. Pneumococci or other mouse-virulent organisms may be standardized according to a mouse-protection index.

3. Attainment of higher protective indexes by variations in test conditions is meaningless. It is far more important to determine, under standardized conditions, that each lot is as good as those preceding.

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A Study of the Repeated Administration of Tinnevelly and Alexandria Senna to Mice*

By MARIBELLE WOODS and I. W. GROTE

To determine if the same mice could be used repeatedly over long periods of time without change in sensitivity, two sets of experiments were conducted. In the first set of experiments no tolerance to either Tinnevelly or Alexandria senna developed. In the second set of experiments, 31 out of 40 mice survived the twenty-three-week period. The average percentage laxative response for the period was 64 per cent (range 38–92 per cent). The responses of individual subjects were roughly random. It would therefore seem that mice may be used once a week for quantitative assay of laxative activity of senna.

A STANDARDIZED PROCEDURE for determining the laxative activity in mice of senna preparations has been previously reported (1). The finely ground drug was suspended in boiling distilled water and 0.5-cc. doses were given by stomach tube. The results were considered positive if the feces were softened sufficiently to stain the underside of newsprint paper, and negative if no stain was visible. To determine if the same mice could be given a weekly dose of senna over long periods of time without changing their sensitivity to the drug, two sets of experiments were conducted.

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EXPERIMENTAL

In the first set of experiments, two groups of 25 male mice each were individually numbered and a record was kept, week by week, of each animal's response to senna and his weight. Tinnevelly and Alexandria senna were given on alternate weeks at three dosage levels, namely 8.3, 6.3, or 5.0 mg. That is, one week group I would receive 8.3 mg. of Tinnevelly senna, while group II would receive the same dosage of Alexandria. The following week the two groups would receive the same dosage as the first week, but the kinds of senna were reversed for the groups. This procedure was followed in succeeding weeks until each group had received three different doses of two kinds of senna. Then this identical procedure was repeated so that each group again received the same dosage and kind of senna which had been received six weeks before. This was to determine whether the five weekly doses which had intervened had changed the mouse's

TABLE I.—EFFECT OF TINNEVELLY AND ALEXANDRIA SENNA ON TWO GROUPS OF 25 MALE MICE

	8.3 Mg. No. Pos./ No. Mice	6.3 Mg. No. Pos./ No. Mice	5.0 Mg. No. Pos./ No. Mice	M. La. D_{50},* Mg.
Group I				
Tinnevelly				
First response	23/25	18/24	13/23	4.5
Same dosage after 6 weeks	24/24	21/24	12/21	4.7
Alexandria				
First response	23/25	8/24	11/24	6.1
Same dosage after 6 weeks	19/24	17/24	7/22	5.6
Group II				
Alexandria				
First response	20/25	11/23	9/22	5.9
Same dosage after 6 weeks	19/22	12/22	8/21	5.8
Tinnevelly				
First response	22/24	15/22	15/22	4.4
Same dosage after 6 weeks	21/22	14/22	11/21	4.9

* M. La. D_{50}—Minimum dose causing laxation in 50% animals tested.

TABLE II.—EFFECT OF WEEKLY ADMINISTRATION OF 6.25 MG. TINNEVELLY SENNA TO 40 ADULT MALE MICE

Date	Weather	Humidity	Temperature	Av. Weight	No. Pos./ No. Mice	Percentage Positive
2/23/44	Sunny	36	77	18.9	31/40	77.5
3/1/44	Sunny	40	74	20.9	33/40	82.5
3/8/44	Sunny	34	62	21.7	18/39	46.1
3/15/44	Sunny	42	74	23.0	15/39	38.5
3/22/44	Cloudy	48	74	23.9	26/39	66.6
3/29/44	Cloudy	42	70	24.6	28/39	71.8
4/5/44	Sunny	30	72	24.4	24/37	64.8
4/12/44	Sunny	44	70	25.8	32/35	91.5
4/19/44	Sunny	54	70	25.9	24/35	68.6
4/26/44	Rain	52	70	26.4	23/35	65.7
5/3/44	Cloudy	45	76	25.4	26/35	74.3
5/10/44	Sunny	46	75	25.5	15/35	42.9
5/17/44	Sunny	48	82	26.9	13/34	38.2
5/24/44	Showers	50	80	27.4	29/34	85.3
5/31/44	Sunny	48	82	27.3	17/34	50.0
6/7/44	Sunny	36	70	26.7	21/34	60.6
6/14/44	Showers			26.8	30/33	90.1
6/21/44	Sunny	32	84	26.8	26/33	76.8
6/28/44	Sunny	42	84	27.2	21/33	63.6
7/6/44	Cloudy			27.0	19/33	57.5
7/12/44	Cloudy	54	80	26.9	12/32	38.1
7/19/44	Showers	60	78	26.8	19/32	59.4
7/27/44	Sunny	40	84	27.1	21/31	67.7

sensitivity to kind and dosage of drug. At the beginning of the experiment, each of the two groups had an average weight of 18 Gm. (range 15–22 Gm.). At the close of the experiment, the average weight for each group was 24.5 Gm. (20–29 Gm. range for group I and 21–31 Gm. range for group II). From the results which are shown in Table I we conclude that:

(*a*) The two groups of 25 mice each agree in average laxative response within 10%.

(*b*) The particular sample of Alexandria senna used in this test was 70% as active as the particular sample of Tinnevelly senna.

(*c*) No development of tolerance to senna was noted during the twelve-week experimental period.

(*d*) An average gain of 6.5 Gm. per mouse during the test period did not alter the mouse's response to constant senna doses.

(*e*) Only one mouse in each group showed positive laxation at all three dosage levels.

In the second set of experiments 40 adult male mice were individually numbered and weighed, so that the response from each mouse could be tabulated week after week. Weekly doses of 6.25 mg. of our standard senna in 0.5-cc. distilled water were given for a period of twenty-three weeks. This dose was chosen as one which might be expected to give a positive laxative effect in 50–70% of the mice tested. The results are given in Table II, from these we conclude that:

(*a*) Each week the group of mice varied above and below the mean in response to a fixed dose of 6.25 mg. senna, but that no tolerance or sensitization due to the weekly dose of senna developed.

(*b*) Variation in response to the same senna dosage ranged from 38.1 to 91.5% for the group, averaging 64.2% for the twenty-three week test period.

(*c*) Each individual animal statistically, therefore, should have shown a positive response 16 times, and a negative response 8 times. Actually, of the 31 survivors, 21 animals, or 68%, gave 13–19 responses (20% variation from the expected figure), and 25 animals, or 80%, gave 12–20 responses

TABLE III.—WEEKLY WEIGHT (IN GRAMS) AND RESPONSE OF INDIVIDUAL MICE TO VARYING DOSES OF TINNEVELLY AND ALEXANDRIA SENNA. FEBRUARY 23 TO MAY 10, 1944. GROUP I

Date:	2/23	3/1	3/8	3/15	3/22	3/29	4/5	4/12	4/19	4/26	5/3	5/10
Sample:	T*	A	T	A	T	A	T	A	T	A	T	A
Dose, Mg.:	8.3	8.3	6.3	6.3	5.0	5.0	8.3	8.3	6.3	6.3	5.0	5.0
Mouse No.												
1	18 +	19 +	20 +	24 +	25 +	24 +	23 +	25 +	24 +	24 +	26 +	25 +
2	19 +	22 +	20 +	22	22	23 +	23 +	24 +	24 +	23	25 +	20
3	21 +	22 +	22 +	23	23	25 +	25 +	27 +	25 +	28 +	28	28
4	19 +	20 +	23 +	26	24 +	25	26 +	26	26 +	28 +	28 +	28 +
5	18 +	20 +	23	22	21	21	22 +	24	24 +	26 +	25	23
6	19 +	20 +	21 +	24	25 +	26 +	26 +	27 +	28 +	28 +	29 +	28
7	18 +	20 +	20 +	22	23	24	24 +	26 +	24 +	26 +	25 +	24
8	17 +	20 +	19 +	25 +	25	26 +	25 +	25 +	25 +	28 +	28 +	27
9	16 +	17 +	18	23	23 +	24	23 +	24 +	22 +	23 +	23 +	21
10	19 +	20 +										
11	19 +	20	23	25 +	16 +	17	16 +	19 +	18 +	20	21	21
12	16 +	16 +	17 +	21 +	22 +	22 +	22 +	22	22 +	24 +		
13	16 +	17 +	18 +	20	20	21	19 +	21 +	21	23	23	23
14	15 +	16 +	18	22 +	20 +	21 +	21 +	22 +	23 +	24 +	23	23
15	18 +	20 +	19 +	22	22 +	23 +	21 +	20	18 +	18 +	20 +	20 +
16	17 +	18 +	19	21	21	20 +	19 +	20 +	21 +	24 +	26	25
17	17 +	19 +	19	22	23	24	24 +	25 +	25 +	25 +	25 +	25
18	18 +	18 +	18 +	22	22 +	23	22 +	22 +	22	23 +	24 +	24 +
19	22 +	23 +	25 +	28		28 +	29 +	30 +	28 +	21		
20	17	15 +	18 +	20 +	22	24	24 +	25	26	26		27
21	19 +	20	22 +	23 +	24 +	25 +	21 +	22 +	24 +	26 +	27	26 +
22	17 +	18 +	18 +	20	20 +	21	20 +	23 +	23 +	25 +	25	24 +
23	18	19 +	19 +	22 +	23 +	24	24 +	23 +	22 +	22	22 +	21
24	20 +	21 +	21 +	25	25 +	26	26 +	28 +	29 +	30 +	32 +	29 +
25	18 +	19 +	20 +	22	21	22	23 +	25 +	23 +	24	23	24

* T—Tinnevelly senna. A—Alexandria senna.
Positive response is indicated with + sign. Negative response is left unmarked.

TABLE IV.—WEEKLY WEIGHT (IN GRAMS) AND RESPONSE OF INDIVIDUAL MICE TO VARYING DOSES OF TINNEVELLY AND ALEXANDRIA SENNA. FEBRUARY 23 TO MAY 10, 1944. GROUP II

Date:	2/23	3/1	3/8	3/15	3/22	3/29	4/5	4/12	4/19	4/26	5/3	5/10
Sample:	A*	T	A	T	A	T	A	T	A	T	A	T
Dose, Mg.:	8.3	8.3	6.3	6.3	5.0	5.0	8.3	8.3	6.3	6.3	5.0	5.0
Mouse No.												
1	15	12										
2	15 +	15 +	15 +	17 +	18	19 +	19 +	20	22	22	21	22 +
3	18	20 +	19	23	21 +	22 +	21 +	23 +	22 +	24	24	24
4	18 +	19 +	20	24 +	25	27 +	26 +	29 +	29	31	30	31
5	20	22 +	23	26	25	27	27 +	29 +	30 +	30 +	31	29 +
6	19 +	21 +	20 +	24 +	25 +	25 +	25 +	27 +	27	28 +	27	26
7	21 +	21 +	22	25 +	25	24 +	24 +	25 +	24 +	26 +	26 +	26 +
8	18 +	18 +	16 +	19 +	18	20 +	21 +	24 +	23	25 +	26	26
9	20 +	20 +	21	23	21 +	21 +	21 +	22 +	23 +	25 +	26	25
10	20 +	21 +	21	24	23	23	23 +	24 +	24	26	27	27
11	17 +	18 +	18 +									
12	22 +											
13	17 +	18 +	18 +	21 +	20 +	22	22	23 +	23 +	24	25	25 +
14	15	18	20	22	21	22 +	23 +	25 +	25	26 +	27 +	25
15	19 +	19 +	16	16 +	16	18	20 +	20 +	21	22 +	23 +	22 +
16	15 +	17 +	17 +	20 +	19 +	20 +	20	22 +	20 +	23 +	21 +	23
17	19	21 +	20	22 +	20	20	20	23 +	21	19		
18	17 +	18 +	17 +	20 +	19	20 +	19 +	21 +	20	18	21	21
19	20 +	21 +	22	25 +	25 +	25 +	25 +	26 +	25 +	26 +	26	26 +
20	20 +	20 +	22 +	24	24 +	24	25 +	26 +	25 +	25 +	26	25 +
21	15 +	17 +	18 +	21	20 +	21 +	20 +	21 +	21 +	21 +	23 +	23 +
22	19 +	20 +	17 +	20 +	19 +	19 +	17 +	20 +	21 +	24 +	26 +	23 +
23	15 +	16 +	16	19 +	18	19	20 +	22 +	22 +	22 +	23 +	21 +
24	16 +	15 +	13	15 +	15	17 +	19 +	19 +	21	23	24	23
25	18 +	20 +	19 +	18 +	19	21 +	18 +	22 +	22 +	22 +	22 +	22 +

* A—Alexandria senna. T—Tinnevelly senna.
Positive response is indicated with + sign. Negative response is left unmarked.

TABLE V.—WEEKLY WEIGHT AND RESPONSE OF INDIVIDUAL MICE TO 6.25 MG. TINNEVELLY SENNA. FEBRUARY 23 THROUGH JULY 27, 1944[a]

Date: / Mouse No.	2/23	3/1	3/8	3/15	3/22	3/29	4/5	4/12	4/19	4/26	5/3	5/10	5/17	5/24	5/31	6/7	6/14	6/21	6/28	7/6	7/12	7/19	7/27
1	20	22 +	23 +	24 +	24	27 +	27	27 +	29 +	30	29 +	28 +	30	32 +	31 +	30 +	30 +	31	31	32	32	33	32 +
2	21 +	23 +	25	27	27 +	27	28	31 +	31	29 +	30 +	31	34	34 +	34 +	34	35	36 +	35 +	35	35	33 +	35
3	19 +	21 +	22	22	23	24 +	25	26 +	27	27	26	26	28	30 +	30 +	29	29 +	30 +	30	30	31	30	32 +
4	19 +	21 +	21 +	22	22 +	24 +	24 +	25 +	25	26	25 +	26 +	27 +	23 +	22 +	21 +	22 +	23 +	24	24 +	24	25 +	24 +
5	19 +	20 +	21	23	23 +	24 +	23 +	24 +	24 +	25 +	24 +	24 +	26	26 +	26 +	24 +	23 +	23 +	22 +	21 +	20 +	24	25
6	19	22 +	22 +	24	25	26	22 +	23 +	25 +	23 +	25 +	24	25 +	26	27	25 +	25 +	28 +	26 +	26 +	27 +	28 +	27 +
7	20	24	24	27	28	29 +	30 +	31 +	32	31 +	31	32	33 +	32 +	34	34	32 +	30	27	28	27	26	25
8	17 +	19 +	20	21	22	22 +	22	23 +	24 +	25	23	24	26	26 +	26	25	25 +	26	26	26	24 +	20 +	19 +
9	17 +	20 +	21 +	23 +	24 +	25 +	26 +	27 +	27 +	28 +	27 +	27	29	31	30 +	29	30 +	30 +	30	30 +	30	29	31
10	21 +	23 +	23 +	25 +	26 +	27 +	26 +	27 +	28 +	29	27 +	28 +	30 +	31 +	30 +	30 +	30 +	30 +	30 +	29 +	28	28 +	30
11	17 +	18 +	19 +	19	20	21 +	22	23 +	23 +	23	23 +	24 +	25 +	25 +	25 +	25 +	25 +	26	26 +	26	26	25	
12	19 +	20 +	23	24 +	25 +	26 +	18																
13	17	19 +	18	20	20 +	22	23	23 +	24 +	25	24 +	25	26	26 +	25	25	26 +	26 +	26	28	27	26 +	27 +
14	18 +	21 +	21 +	23 +	24 +	25 +	25 +	27 +	26	26	24	27	29 +	29 +	29	29 +	30 +	29 +	30	29 +	30 +	31 +	30 +
15	20 +	21 +	22	22	23	24 +	24 +	24 +	24 +	25 +	22 +	22 +											
16	19 +	21 +	23 +	25	24	26 +	26 +	27 +	28 +	27 +	27	28	30 +	30 +	29	28 +	29 +	30 +	28 +	30 +	29	27 +	26 +
17	19 +	21 +	22	23	24 +	25 +	25	25 +	22 +	23 +	21	21	23 +	23 +	24	24	24 +	24 +	23 +	21 +	26	21 +	21 +
18	18 +	21	20	23	25 +	26 +	27 +	27 +	27 +	28 +	26 +	26 +	27	29 +	28	28 +	28	29 +	30 +	30	29	28	30 +
19	18 +	20 +	21	22	23	25	25 +	22 +	21 +	23 +	22 +	21	23 +	22 +	24	24 +	24 +	24 +	24 +	24	25	25	26
20	18 +	19 +																					
21	18 +	21 +	20 +	18 +	20	21 +	21 +	23 +	23	24 +	23 +	23 +	23	25 +	25	26 +	24 +	25 +	26	25	26 +	23 +	22 +
22	18 +	20	20	21	22 +	24 +	24	25 +	25 +	26 +	26 +	26	27	26 +	26	24 +	24 +	24 +	24	24 +	22	21	23
23	19 +	21 +	22 +	24	24 +	26	22 +	25 +	26	25 +	23 +	23	24 +	25 +	26 +	26	28 +	27 +	26 +	28 +	29 +	28 +	29 +
24	21 +	22 +	23	25	27	18																	
25	18	20 +	20 +	22	23 +	24 +	23	25 +	24 +	25 +	25 +	24	24	28	26	26	25 +	25 +	26 +	25	25	26	27 +
26	18 +	20 +	21 +	23 +	24 +	26 +	25 +	26 +	24 +	24 +	22 +	21 +	22	23 +	23 +	20 +	20 +	20 +					
27	18 +	18 +	19 +	18 +	15 +	16																	
28	18 +	20 +	21 +	21 +	21 +	23 +	23 +	25 +	25 +	24 +	21	21 +	22	22 +	24 +	24 +	24 +	25 +	25 +	25 +	25 +	25 +	25 +
29	19	21 +	21	23	25	26 +	26 +	28 +	28 +	30 +	29 +	28	30 +	30 +	31	26	23 +	24 +	24 +	20 +			
30	21 +	23 +	25	27	26 +	27	26	28	27 +	29	29 +	28	30	31	30	30	30 +	32	30	32	33		
31	18	21 +	21 +	24 +	26 +	27 +	27 +	26	27	29 +	27 +	29 +	31	30 +	28 +	27 +	29 +	27 +	27 +	25 +	25 +	25 +	25 +
32	19 +	21 +	22	23	24 +	25 +	25 +	27 +	26	27 +	26 +	25	22 +	21 +	23	23 +	22	24 +	24 +	25 +	25	24	24
33	18 +	20 +	20	22 +	24 +	23	21 +	24	23 +	24 +	23	23	23	27	26 +	25 +			25 +	25 +	25	25 +	25 +
34	21	23	24	25	27 +	24 +	27	29 +	30	30	29	29	30	32 +	30 +	31	34 +	34 +	32	30	30	27 +	25 +
35	21 +	22	22 +	23 +	25 +	22	24 +	25 +	25 +	26 +	27 +	25 +	25	24 +	25 +	24 +	27 +	24		29 +	29 +	29 +	24 +
36	18 +	18 +	20 +	21 +	22 +	23 +	23 +	24 +	24 +	25 +	23 +	24 +	26	26 +	25 +	25 +	25 +	24 +	25 +	24 +	25 +	25 +	29 +
37	22 +	25 +	26 +	26	27	28 +	28 +	29 +	29 +	30	28 +	28 +	30	31 +	30	30 +	30 +	22 +	31	31	31 +	33	32
38	18	18	19	22	24 +	24 +	23 +	25 +	25	26	26 +	24	25	26 +	26	26	25 +	24 +	24 +	23 +	23 +	24 +	24 +
39	20 +	23	24	26 +	28 +	29	20																
40	21 +	23 +	25	26 +	26 +	27 +	27 +	28 +	27 +	29 +	27 +	28 +	30 +	30 +	30 +	30 +	30 +	30	33 +	31 +	30	32 +	33 +

[a] Positive response is indicated with + sign. A negative response is unmarked.

(25% variation from the expected figure). The responses would therefore seem to be roughly random, with a fairly high correlation at the expected level.

CONCLUSIONS

In conclusion it would seem that:

1. Mice, like men, show random variation in laxative action to the same drug when tested week after week.

2. No tolerance developed to repeated administration of senna from week to week during a twelve- to twenty-three week period.

3. The mouse assay, as carried out in this laboratory, is a quantitative measurement of the laxative strength of senna, and has a probable accuracy of some ±10–15 per cent.

REFERENCE

(1) Grote, I. W., and Woods, M., THIS JOURNAL, 33, 266(1944).

Local Anesthetics. I. Phenylurethanes of Dialkylamino Alcohols*

By F. J. BANDELIN and J. V. TUSCHHOFF

Phenylurethanes of series of 2-dialkylaminoethanols, 1-dialkylamino-2-propanols and 3-dialkylaminopropanols produce local anesthesia which increases in duration with the molecular weight of the amino alkyl groups, the most active of the compounds tested, in each series, being the dibutyl derivative. Higher homologs are too insoluble to be evaluated by the methods described. Test results obtained with cocaine and procaine are included for comparison.

THE PREPONDERANCE of investigation in the synthesis and testing of local anesthetic compounds has been limited to the esters of benzoic or *p*-aminobenzoic acids. Numerous other organic acids may be utilized in place of these and, although not as extensively investigated, many of the latter offer attractive possibilities. One of the most interesting is phenylcarbamic acid, which confers conspicuous topical anesthetic activity upon aminoesters derived from it. Early work (1) with phenylcarbamates, or phenylurethanes, led to the belief that they had rather unsatisfactory properties and consequently the investigation of these compounds lay dormant for a time. This field was reopened by Rider (2–4), who studied the phenylurethanes of dialkylaminopropanediols, and by Cook and Rider (5), who investigated several phenylurethanes of primary and secondary aminopropyl alcohols.

* Received October 2, 1950, from the Research Laboratories of Flint, Eaton & Co., Decatur, Ill.

More recent studies on phenylurethanes of monoalkylaminoethanols (6) and 2-methyl-2-monoalkylaminopropanols (7) have indicated that these compounds possess marked local anesthetic activity. Rau and Westfall (8) have reported the anesthetic activity of some procaine-substituted urethanes. These seem to have activity comparable to or somewhat greater than procaine. To date, however, no systematic study of the phenylurethanes of 2-dialkylaminoethanols, 1-dialkylamino-2-propanols, and 3-dialkylaminopropanols have appeared. The preparation of these compounds was therefore undertaken to investigate their relative anesthetic properties. These data are given in Table I.

SYNTHESIS

It was found expedient to prepare the compounds by one of the following two methods:

Procedure A.—1-Piperidino-2-propylphenylurethane HCl, $C_6H_5NHCOOCH(CH_3)CH_2NC_5H_{10}\cdot HCl$. To a mixture of 25.67 Gm. (0.33 mole) of piperidine and 31.3 Gm. (0.33 mole) of propylene chlorohydrin in 100 ml. of benzene was added 200 ml. of alcoholic potassium hydroxide, and the resulting mixture refluxed for several hours. The reaction mixture was filtered and the filtrate was heated on a steam bath to remove the ethanol and benzene. The residue was distilled at reduced pressure, the 1-piperidino-2-propanol distilling at 73–75° at 7 mm. A yield of 34 Gm., 85% of the amino alcohol was obtained.

The phenylurethane was prepared by dissolving 34 Gm. (0.25 mole) of the amino alcohol in 100 ml.

TABLE I.—PHENYLURETHANES OF AMINO ALCOHOLS

Compound R=	Formula	M. P., °C., Uncorr.	Nitrogen, % Calcd.	Nitrogen, % Found	Chlorine, % Calcd.	Chlorine, % Found	Duration of Anesthesia, Min.— Rabbit Cornea	Duration of Anesthesia, Min.— Human Forearm Wheals
Phenylurethane Hydrochlorides of 2-Dialkylaminoethanols $C_6H_5NHCOOCH_2CH_2R \cdot HCl$								
Dimethylamino[a]	$C_{11}H_{17}N_2O_2Cl$	159–160	11.44	11.40	14.38	14.12	12	11
Diethylamino[a, c, d]	$C_{12}H_{21}N_2O_2Cl$	137–138	10.26	10.13	13.05	12.75	15	24
Dipropylamine[b]	$C_{15}H_{25}N_2O_2Cl$	127–128	9.26	9.35	11.75	11.42	18	34
Diisopropylamino[b]	$C_{15}H_{25}N_2O_2Cl$	145–146	9.26	9.35	11.75	11.46	45	25
Dibutylamino[a]	$C_{17}H_{29}N_2O_2Cl$	135–136	8.48	8.44	10.73	10.71	89	330
Diisobutylamino[b]	$C_{17}H_{29}N_2O_2Cl$	Oil	8.44	10.71	10.73	10.61	1	1
Diamylamino[b]	$C_{19}H_{33}N_2O_2Cl$	Wax	7.81	7.79	9.90	9.54	1	1
Diisoamylamino[b]	$C_{19}H_{33}N_2O_2Cl$	Oil	7.81	7.74	9.90	9.73	1	1
Dihexylamino[b]	$C_{21}H_{37}N_2O_2Cl$	Oil	7.24	7.16	9.17	9.01	1	1
Diisooctylamino[b]	$C_{25}H_{45}N_2O_2Cl$	Oil	6.32	6.29	8.22	8.32	1	1
Piperidino[a]	$C_{14}H_{21}N_2O_2Cl$	211–212	9.81	9.79	12.45	12.43	Inc.	24
Morpholino[a]	$C_{13}H_{19}N_2O_3Cl$	233–234	9.74	9.71	12.32	12.19	Inc.	9
Phenylurethane Hydrochlorides of 3-Dialkylaminopropanols $C_6H_5NHCOOCH_2CH_2CH_2R \cdot HCl$								
Diethylamino[b, d]	$C_{14}H_{23}N_2O_2Cl$	135–136	9.76	9.70	12.39	12.36	Inc.	29
Dipropylamino[b, d]	$C_{16}H_{27}N_2O_2Cl$	158–159	8.84	8.81	11.20	10.91	10	18
Diisopropylamino[b]	$C_{16}H_{27}N_2O_2Cl$	135–136	8.84	8.83	11.20	11.10	1	1
Dibutylamino[b, c, d]	$C_{18}H_{31}N_2O_2Cl$	120–121	8.12	8.16	10.30	10.21	72	315
Diisobutylamino[b]	$C_{18}H_{31}N_2O_2Cl$	Oil	8.12	8.19	10.30	10.13	1	1
Diamylamino[b]	$C_{20}H_{35}N_2O_2Cl$	Wax	7.52	7.46	9.42	9.36	1	1
Diisoamylamino[b]	$C_{20}H_{35}N_2O_2Cl$	Oil	7.52	7.48	9.52	9.41	1	1
Dihexylamino[b]	$C_{22}H_{39}N_2O_2Cl$	Oil	7.00	6.98	8.88	8.64	1	1
Diisooctylamino[b]	$C_{26}H_{47}N_2O_2Cl$	Oil	6.13	6.15	7.77	7.59	1	1
Piperidino[b, d]	$C_{15}H_{25}N_2O_2Cl$	168–169	9.36	9.31	11.82	11.63	Inc.	10
Morpholino[b]	$C_{14}H_{23}N_2O_3Cl$	187–188	9.28	9.28	11.78	11.68	Inc.	7
Phenylurethane Hydrochlorides of 1-Dialkylamino-2-propanols $C_6H_5NHCOOCH(CH_3)CH_2R \cdot HCl$								
Diethylamino[a, d]	$C_{14}H_{23}N_2O_2Cl$	134–135	9.76	9.71	12.39	12.26	Inc.	9
Dipropylamino[b]	$C_{16}H_{27}N_2O_2Cl$	114–115	8.84	8.82	11.20	11.19	20	22
Diisopropylamino[a]	$C_{16}H_{27}N_2O_2Cl$	Oil	8.84	8.81	11.20	11.19	1	...
Dibutylamino[b]	$C_{18}H_{31}N_2O_2Cl$	108–109	8.12	8.14	10.30	10.25	63	55
Diisobutylamino[b]	$C_{18}H_{31}N_2O_2Cl$	Oil	8.12	8.16	10.30	10.15	1	1
Diamylamino[b]	$C_{20}H_{35}N_2O_2Cl$	Oil	7.52	7.43	9.52	9.31	1	1
Diisoamylamino[b]	$C_{20}H_{35}N_2O_2Cl$	Oil	7.52	7.33	9.52	9.24	1	1
Dihexylamino[b]	$C_{22}H_{39}N_2O_2Cl$	Oil	7.00	6.89	8.88	8.76	1	1
Diisoctylamino[b]	$C_{26}H_{47}N_2O_2Cl$	Oil	6.13	6.09	7.77	7.79	1	1
Piperidino[a]	$C_{15}H_{25}N_2O_2Cl$	205–206	9.36	9.33	11.82	11.67	14	14
Morpholino[a]	$C_{14}H_{23}N_2O_3Cl$	182–183	9.28	9.21	11.78	11.71	Inc.	14
Cocaine hydrochloride	.....	...	...	...	...	...	7	...
Procaine hydrochloride	.....	...	...	...	...	...	...	25

[a] Indicates compounds prepared by procedure A.
[b] Indicates compounds prepared by procedure B.
[c] Rider, T. H., *J. Am. Chem. Soc.*, **52,** 2583(1930).
[d] Cook, E. S., and Rider, T. H., *ibid.*, **58,** 1079(1936).

of anhydrous ether, and to this solution was added 30.2 Gm. (0.25 mole) of phenylisocyanate, and the resulting solution refluxed for four hours. The reaction mixture was evaporated to one-half its volume and an equal volume of petroleum ether was added. Dry hydrogen chloride was bubbled through this solution, thus precipitating the 1-piperidino-2-propyl phenylurethane HCl as an amber-colored oil which crystallized on cooling in a refrigerator. The hydrochloride was recrystallized from a mixture of acetone and petroleum ether, m. p. 105–106°; yield 45 Gm., 65%.

Procedure B.—3-Piperidinopropylphenylurethane hydrochloride, $C_6H_5NHCOOCH_2CH_2CH_2NC_5H_{10} \cdot HCl$. An amount (17.2 Gm. or 0.2 mole) of piperidine was heated in a three-neck round-bottom flask equipped with a reflux condenser, a dropping funnel, and a mechanical stirrer. To the boiling piperidine, 18.9 Gm. (0.2 mole) of trimethylenechlorohydrin was added dropwise and with constant stirring. The mixture was refluxed for thirty minutes after the last of the trimethylenechlorohydrin had been added, at which time 100 ml. of alcoholic KOH was added and the refluxing continued for an additional eight hours. The reaction mixture was cooled, the KCl removed by filtration, and the ethanol removed by distillation. The residue was distilled at reduced pressure, the 3-piperidinopropanol distilling a 74–77° at 1 mm. A yield of 16 Gm., 60% of the amino alcohol, was obtained.

The phenylurethane was prepared by dissolving 16 Gm. (0.12 mole) of 3-piperidinopropanol in 50 ml. of anhydrous ethyl ether, and to this solution was added 14.3 Gm. (0.12 mole) of phenylisocyanate, and the resulting mixture was evaporated to one-half its volume and an equal volume of petroleum ether was added. Dry hydrogen chloride was bubbled through the solution thus precipitating the 3-piperidinopropylphenylurethane hydrochloride as a white crystalline compound which was

filtered off and recrystallized from a mixture of acetone and petroleum ether, m. p. 168–169°; yield 23.4 Gm., 65%.[1-4]

METHODS OF TESTING

Measuring Surface Anesthesia.—This was accomplished by applying 0.5% solutions of compounds to the rabbit's cornea in a continuous stream from a wash bottle in such a manner that the total surface area of the eye was covered with solution for a period of one minute. The cornea was then touched lightly with the point of a pencil, and the presence or absence of the wink reflex noted. The duration of anesthesia was measured from the disappearance of the wink reflex to its return.

Measuring Infiltration Anesthesia.—Infiltration anesthesia was tested by the wheal method on the planar surface of the human forearm. One-tenth cubic centimeter of 0.5% solution was injected intradermally, and the extent of the raised wheal outlined. Reaction to stimulation of the wheal is the criterion of anesthesia. The marked area was stimulated by pricking with a nylon bristle with the individual blindfolded so that observation did not influence response. Duration of anesthesia was measured from the time of the disappearance of the pricking sensation caused by the nylon bristle until this sensation, at the center of the wheal, returned to the extent that it could again be felt.

The duration of anesthesia produced by each compound and determined by these methods is recorded in minutes, except for those compounds whose limited solubility did not permit evaluation by the above techniques (Table I).

Values derived by these methods for surface anesthesia produced by cocaine hydrochloride and infiltration anesthesia produced by procaine hydrochloride are given for comparison.

DISCUSSION

The topical and intradermal anesthetic activity of these compounds generally increased with the molecular weight of the alkyl groups of the amine alkyls with the greatest increase found at the butyl level. Diisobutyl alkyls or dialkyls greater than butyl are too insoluble for testing by ordinary methods. Of the di-isopropyl compounds, only the di-isopropyl amino ethanol phenylurethane hydrochloride was sufficiently soluble for testing. This compound far exceeded its *n*-propyl analog in anesthetic activity. This coincides with the findings of Schmitz and Loevenhart (9), who found the isopropyl group superior to the *n*-propyl group in the 3-dialkylaminopropanol *p*-aminobenzoate analogs. The dibutyl-aminoethyl and dibutylaminopropyl compounds exhibited durations of anesthesia approximately 13 times that of procaine intradermally, and approximately 13 and 10 times that of cocaine intradermally and approximately 13 and 10 times that of cocaine topically. It is of interest to note that these compounds are active both topically and by infiltration. Furthermore, these compounds produce anesthesia of longer duration than do the corresponding benzoate analogs.

Although some of the lower molecular weight analogs produced considerable stinging and some irritation when injected intradermally, this became less as the molecular weight increased. The dibutyl derivatives produced only slight irritation. Topically, on the rabbit cornea, none of the compounds produced edema, inflammation, or corneal pitting.

A more comprehensive and extended study of these compounds will be published.

SUMMARY

A series of 2-dialkylamino ethanol, 3-dialkylamino propanol, and 1-dialkylamino-2-propanol phenylurethane hydrochlorides have been prepared as described. The duration of anesthesia produced by these compounds was determined by topical application to the rabbit cornea and by intradermal injection into the human forearm of 0.5 per cent solutions. The testing methods used are outlined. Values found for cocaine hydrochloride and procaine hydrochloride are given for the purpose of comparison. In each of the three series, the dibutyl compound was, by far, the most active. Testing of the higher molecular weight homologs by the methods employed was not possible because of their relative insolubility.

[1] Amino alcohols prepared from the lower molecular weight amines were, in most instances, best prepared by procedure A, which gave good yields. Higher molecular weight and branched dialkylamines required more drastic conditions for reaction, and were best prepared by procedure B.

[2] Most of the phenylurethane hydrochlorides could be recrystallized from a mixture of acetone and petroleum ether. In a few cases, ethyl ether and benzene mixtures were used. If this failed, the compound usually failed to crystallize under any conditions and remained an oil.

[3] In preparing the di-isoalkylamino alcohols by procedure B it was found necessary, in order to obtain good yields, to double or triple the refluxing time both before and after the addition of alcoholic potassium hydroxide.

[4] A third procedure for the preparation of these compounds was tried. This consisted of reacting the phenylisocyanate with the halohydrin, thus forming the chloroalkylphenylcarbamate. After distillation of the carbamate it was reacted with the various secondary amines. Yields of the various compounds prepared by this method were much lower than with methods A and B, respectively.

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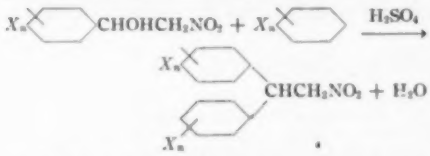
Antibacterial Properties of a Series of Diarylalkylammonium Chlorides*,†

By E. B. HODGE, M. C. BACHMAN, and M. B. NEHER‡

The preparation and properties (including bacteriostatic activity) of a series of diarylalkylammonium chlorides are described. Several of these compounds are active bacteriostatic agents with wide bacterial spectrums. Small changes in the structures of the compounds in the series cause marked changes in antibacterial activity.

CONDENSATIONS between 1-aryl-2-nitroethanols and some aromatic compounds have been disclosed by Müller (1). These reactions take place as shown in the equation below:



where X = halogen, hydrogen, or methyl; $n = 1$ or 2. Extension of this reaction by Hass and Neher (2), by Hass, Neher, and Blickenstaff (3), and by the senior author has made available an extensive series of diarylalkyl nitro compounds

It has been found that the hydrochlorides of the amino compounds formed by reducing some of these nitro compounds have pronounced antibacterial activity, and it has been found possible to correlate their structures and antibacterial activities to a certain extent

The formulas, melting points, and analyses of the series of amine hydrochlorides formed as indicated above are given in Table I, and the bacteriostatic activities of these compounds are shown in Table II.

In order to study further the relationship between structure and activity in compounds of this type, N,N-dimethyl-2-*o*-chlorophenyl-2-*p*-chlorophenylisopropylammonium chloride and 1,1-bis-(*p*-chlorophenyl)-2-acetamidopropane were prepared and tested. These two compounds showed no antibacterial activity under the conditions of the test. Likewise 2-nitro-1,1-bis-(*p*-chlorophenyl)propane, the parent compound of one of the most active amine hydrochlorides showed no antibacterial activity.

It is evident that slight changes in structure in compounds of the type studied cause marked changes in antibacterial activity. The introduction of substituents on the aromatic groups causes changes in antibacterial activity which vary with the nature and position of the substituents. In general, chlorine seems to be one of the most effective substituents for increasing antibacterial activity.

The organisms selected represent a rather wide variety of types and compounds such as 2,2-bis-(*p*-chlorophenyl)isopropylammonium chloride, which showed activity against every organism tested, would be expected to have a rather wide bacterial spectrum. This compound (P-21) was selected for further tests. It was found to be irritating when injected subcutaneously into guinea pigs in amounts as low as 20 mg. per day and was not tested further *in vivo*. Since it appeared that the most likely field of application of P-21 would be as a surface antiseptic, further tests were made to explore this possibility. Without going into detail on the results of these tests, they can be summarized by the statement that P-21 is very similar both in bacteriostatic and bactericidal activity to at least two widely used quaternary ammonium compounds, but in a few cases is slightly less active than these compounds.

EXPERIMENTAL

Preparation of Amine Hydrochlorides.—The amine hydrochlorides were all prepared in good yields by substantially the same procedure. Their preparation will be illustrated by a description of the conditions used for the preparation of 2,2-bis-(*p*-chlorophenyl)isopropylammonium chloride.

A solution of 75 Gm. (0.242 mole) of 2-nitro-1,1-bis(*p*-chlorophenyl)propane in 800 ml. of methanol was reduced at 40° and 1,500 lb. per square inch hydrogen pressure in the presence of 15 Gm. of Raney nickel for about six hours. Then the catalyst was filtered out, 30 ml. of concentrated hydrochloric acid was added, and the solution was concentrated until crystals appeared. It was then cooled, stirred with 500 ml. of cyclohexane to give 60 Gm. (78%) of nearly white product, m. p. 262–266°. The purified product (recrystallized from ethanol-cyclohexane) melted at 269–270° (corr.) with slight decomposition.

Preparation of 2-*o*-Chlorophenyl-2-*p*-chlorophenyl-N,N-dimethylisopropylammonium Chloride.—Fifty-eight grams (0.183 mole) of 2-*o*-chlorophenyl-2-*p*-chlorophenylisopropylammonium chloride was

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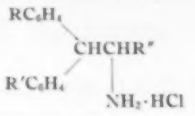
† Presented before the Division of Medicinal Chemistry, American Chemical Society, Philadelphia, April 11, 1950.
‡ Purdue University. Present address: Department of Chemistry, Ohio University, Athens, Ohio.

TABLE I.—MELTING POINTS AND ANALYSES OF A SERIES OF AMINE HYDROCHLORIDES,



Compound No.	R	R′	R″	M. p.[a] (° C.)	Analyses, %: Chlorine Calcd.	Chlorine Found	Nitrogen Calcd.	Nitrogen Found
1	4-Cl	4-Cl	H	227–229	35.15	34.83	4.63	4.48
2	2-Cl	4-Cl	H	292–298 (d)[b]	35.15	35.28	4.63	4.62
3	2-Cl	4-CH(CH$_3$)$_2$	H	224	22.86	22.59	4.51	4.38
4	H	H	CH_3	276–278	14.32	14.61	5.65	5.46
5	H	2,4-DiCH$_3$	CH_3	300–303 (d)	12.86	12.90	5.08	4.88
6	2-Cl	H	CH_3	288–289 (d)	25.13	24.77	4.96	4.90
7	2-Cl	4-F	CH_3	303–304 (d)	23.62	23.23	4.67	4.53
8	2-Cl	4-Br	CH_3	276–282 (d)	19.64	19.25	3.88	3.71
9	2-Cl	4-CH$_3$	CH_3	291–293 (d)	23.94	23.86	4.73	4.63
10	2-Cl	4-Cl	CH_3	285–287 (d)	33.59	33.18	4.42	4.46
11	4-CH$_3$	4-CH$_3$	CH_3	242–247 (d)	12.86	13.02	5.08	5.14
12	4-Cl	4-CH(CH$_3$)$_2$	CH_3	295–300 (d)	21.86	22.18	4.32	4.19
13	4-Cl	4-C$_2$H$_5$	CH_3	244–258 (d)	22.85	22.99	4.51	4.51
14	4-Cl	4-CH$_3$	CH_3	265–267 (d)	23.93	24.13	4.73	4.64
15	4-Cl	4-Cl	CH_3	269–270 (d)	33.59	33.17	4.42	4.40
16	4-Cl	2,4-DiCl	CH_3	289–292 (d)	40.51	40.30	3.99	3.92
17	4-Cl	4-Cl	C_2H_5	225	32.17	31.82	4.24	3.96

[a] All melting points are corrected.
[b] (d) indicates decomposition, which varied from slight yellowing to considerable darkening.

TABLE II.—BACTERIOSTATIC ACTIVITIES OF COMPOUNDS SHOWN IN TABLE I

Compound No.	*S. aureus*[a]	*S. fecalis*[b]	*S. hemolyticus*[c]	*E. coli*[d]	*P. aeruginosa*[e]	*S. dysenteriae*[f]	*M. ranae*[g]	*P. pseudotuberculosis*[h]	*S. paradysenteriae*[i]	*M. Special*[j]
Minimum Inhibitory Concentrations in μg./Ml. Against:										
1	100	75	>100	>100	...	50	...	50	...	25
2	>100	>100	>100	>100	>100	>100	10	...	...	...
3	75	50	75	75	...	...	...	10	50	...
4	>100	>100	>100	>100	...	...	>100	...	>100	>100
5	100	100	100	100	...	...	>100	25	100	...
6	>100	>100	>100	>100	...	100	...	>100	...	...
7	>100	>100	>100	>100	...	...	100	...	>100	100
8	100	100	>100	>100	...	>100	...	>100	...	...
9	100	>100	100	>100	...	>100	...	>100	...	...
10	50	25	50	100	...	...	>100	25	50	...
11	100	100	>100	>100	...	>100	...	>100	...	...
12	100	50	50	100	100	100	10	...	...	...
13	20	50	50	>100	50	50	10	...	...	...
14	75	100	50	75	...	...	100	25	100	...
15	50	50	50	100	50	50	10	...	...	...
16	25	25	25	>100	...	...	...	25	75	...
17	100	75	100	>100	...	>100	...	100	...	...

[a] *Staphylococcus aureus* (A.T.C.C. No. 9144). [b] *Streptococcus fecalis* (A.T.C.C. No. 6057). [c] *Streptococcus hemolyticus* (Lancefield Group O). [d] *Escherichia coli* (A.T.C.C. No. 167). [e] *Pseudomonas aeruginosa* (A.T.C.C. No. 9027). [f] *Shigella dysenteriae* (A.T.C.C. No. 8712). [g] *Mycobacterium ranae* (Kurung). [h] *Pasteurella pseudotuberculosis* (A.T.C.C. No. 6902). [i] *Shigella paradysenteriae* (A.T.C.C. 9199). [j] *Mycobacterium Special* (A.T.C.C. 607).

stirred and heated with a solution of 20 Gm. of sodium hydroxide in 200 ml. of water for thirty minutes. Then the mixture was cooled and extracted with 150 ml. of ether. The ether was dried over sodium sulfate and evaporated. To the residue was added 100 ml. of methanol and 11 Gm. (0.367 mole) of paraformaldehyde. This mixture was boiled for five minutes. It was then diluted with 500 ml. of methanol and reduced at 50° and 1,500 lb. per square inch hydrogen pressure in the presence of 10 Gm. of Raney nickel for five hours. After the catalyst had been filtered and the solvent had been evaporated, the residue was recrystallized from a benzene-ethanol mixture to give 15 Gm. (24%) of white crystals; m. p. (corr.), 211–212°.

Anal.—Calcd. for $C_{17}H_{20}Cl_2N$: Cl, 30.86; N, 4.06. Found: Cl, 30.69; N, 3.88.

Preparation of 1,1-bis-(*p*-Chlorophenyl)-2-acetamidopropane.—To a solution of 3.2 Gm. (0.01 mole) of 2,2-bis-(*p*-chlorophenyl)isopropylammonium chloride in 25 ml. of methanol was added 0.6 Gm. (0.011 mole) of sodium methoxide and then

3 ml. (0.03 mole) of acetic anhydride. The mixture was boiled gently for fifteen minutes, then cooled and poured into 50 ml. of water. Filtration gave 3.1 Gm. (97%) of white crystals, m. p., 170–174°. One recrystallization from cyclohexane, plus a little methanol, gave 2.3 Gm. (72%); m. p. 173–174° (corr.).

Anal.—Calcd. for $C_{17}H_{17}Cl_2NO$: Cl, 22.01; N, 4.35. Found: Cl, 21.70; N, 4.23.

Bacteriostatic Tests.—Each compound to be studied was incorporated in North Gelatin Agar (Difco) at the following levels: 100, 75, 50, 25, 5, 1.0, 0.1, and 0.01 μg./ml. (The highest level used for 1,1-bis-(*p*-chlorophenyl)-2-acetamidopropane was 50 μg./ml.). The plates were then streaked with saline suspensions of the test organisms washed from eighteen-hour agar slants. All plates except those containing mycobacteria were incubated for twenty-four hours at 37° before readings were taken. The acid-fast organisms were incubated for forty-eight hours. The inhibition readings were recorded as the lowest concentration at which no growth of the bacterial strain occurred.

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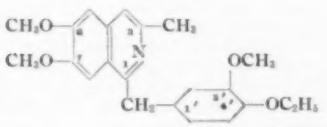
Pharmacologic Studies of 6,7-Dimethoxy-1-(4′-ethoxy-3′-methoxybenzyl)-3-methyl-isoquinoline*

By F. G. HENDERSON, R. E. SHIPLEY, and K. K. CHEN†

The synthetic analog of papaverine, 6,7-dimethoxy-1-(4′-ethoxy-3′-methoxybenzyl)-3-methyl-isoquinoline,[1] appears to be equal to papaverine in ability to dilate coronary and femoral arteries. This is true of the hydrochloride as well as the phosphate. It has very little effect on isolated or intact intestine. Acute toxicity studies show it to be less toxic than papaverine, both by intravenous injection in albino mice and oral administration in albino rats. Chronic toxicity studies failed to show any significant detrimental effect in rats receiving 100 mg. of the drug daily, or over 50 per cent of the LD_{50} daily. It is our belief that this compound warrants clinical trial.

PAPAVERINE is well known for its vasodilator effect. In recent years it has been used in the treatment of coronary artery disease (1), cerebral angiospasm (2), periarteritis nodosa (3), Raynaud's disease (4), thromboangiitis obliterans (5), pulmonary embolism (6), and other diseases involving spasm or obstruction of some portion of the arterial system. Recently our organic chemists have synthesized several compounds similar to papaverine in chemical structure and physiologic activity. One of these showed sufficient activity to warrant further study. Chemically it is 6,7-dimethoxy-1-(4′-ethoxy-3′-methoxybenzyl) - 3 - methyl - isoquinoline. The structural formula is as follows:



Two salts of this compound were prepared, the hydrochloride, m. p. 196–208°, and the phosphate, m. p. 197–199°. Both are water soluble, the phosphate to a greater degree than the hydrochloride. In our pharmacologic studies both salts were used.

PHARMACOLOGIC STUDIES

Effect on the Cardiovascular System

Coronary Artery Flow.—The effect of both salts was studied on the coronary arterial flow in mongrel dogs weighing 9–15 Kg. The animals were anesthetized with pentobarbital sodium by vein, and the thorax opened under artificial respiration. The descending, or circumflex, branch of the left coronary artery was isolated for cannulation. Heparin, Pontamine fast pink, or Paritol A was given intravenously to render the blood noncoagulable. The left carotid artery was cannulated, and connected to an optical recording rotameter (7, 8). From the outflow connection of the rotameter, flexible plastic tubing conducted the blood to a cannula inserted and tied into the peripheral portion of the coronary artery, the central end having been tied immediately prior to cannulation. In this manner the rate of blood flow from the carotid artery to the coronary vascular bed was continuously measured by the rotameter.

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† The authors are deeply indebted to Miss Eva Sommermeyer, Dr. P. N. Harris, and Messrs. R. M. Small, H. E. Roeder, and H. W. Worth for their invaluable assistance in this study.
[1] Eli Lilly and Company has registered Paveril phosphate (Dioxyline phosphate, Lilly) as the trade mark of this compound.

Substances to be tested for dilator activity were injected through a rubber-capped "T" connection proximal to the coronary cannula. The administration of the drug was made intra-arterially, and the response of the coronary vascular bed was observed before the drug reached the general circulation. The amounts injected ranged from 0.05 ml. to 0.20 ml. of a 0.10 to 0.5% solution. The quantity was adjusted so that the coronary flow increased 55–140%, with maximum dilatation occurring within the first minute, and a total duration of detectable response of from three to five minutes. Each compound was tested at least twice in each experiment.

The hydrochloride and the phosphate of the new isoquinoline derivative were each tested in four dogs, and the coronary dilator response compared with that of papaverine hydrochloride and papaverine phosphate. There was no significant difference between the dilator activities of the hydrochlorides and the phosphates of the two compounds.

Compared on the basis of equal amounts injected, 6,7-dimethoxy-1-(4′-ethoxy-3′-methoxybenzyl)-3-methyl-isoquinoline caused coronary dilatation of a degree and duration equal to that of papaverine in four dogs, and 5–25% greater in four other dogs.

Femoral Artery Flow.—Similar experiments were performed on barbitalized dogs to determine the effect on femoral arterial flow. The femoral artery was cannulated in such a manner that the flow could be measured with the rotameter (7, 8). Papaverine hydrochloride and the phosphate of 6,7-dimethoxy-1-(4′-ethoxy-3′-methoxybenzyl)-3-methyl-isoquinoline were compared by injection into the femoral artery. In a dose of 0.1 mg., papaverine hydrochloride and the new compound each increased the femoral arterial flow 23 ml. per minute. A dose of 2.0 mg. of each increased the flow 46 ml. per minute for the latter, and 45 ml. per minute for papaverine hydrochloride. The maximum increase was reached twenty seconds following the injection. At the end of two minutes the flow had returned to normal, with the exception of the higher dose, 2 mg. The new analog and papaverine hydrochloride appear to be equal in their ability to dilate the femoral arteries of dogs, weight for weight.

Effect on Blood Pressure, Heart Rate, and Respiration.—The effect of the phosphate of 6,7-dimethoxy-1-(4′-ethoxy-3′-methoxy-benzyl)-3-methyl-isoquinoline was studied in a series of five barbitalized dogs. Carotid blood pressure, respiration, and electrocardiograms were recorded. Intravenous injection of 10 mg./Kg. resulted in a fall of blood pressure of 44 mm. Hg, with a return to normal in ten minutes. This dose had no effect on respiration. Larger doses caused a more marked fall of blood pressure and an acceleration of respiration. One dog received 20 mg./Kg. by vein, and showed a fall of blood pressure of 60 mm. Hg (Fig. 1), which required approximately two hours to return to normal. This dose also elicited an increase in respiratory rate for a period of approximately ten minutes. Electrocardiograms taken following this dose showed an acceleration of heart beat from 127 to 160 per minute. There was a slight increase in the height of the *P* wave, no significant change in the *P-R* interval, and a slight decrease in the amplitude of the *QRS* complex. The most pronounced change was seen in the *T* wave. Initially inverted, it gradually assumed an upright position (Fig. 1) and finally returned to its original inverted state two hours after injection.

Effect on Smooth Muscle

Isolated Organs.—When the phosphate of the new base was added to the Tyrode's solution in which a strip of rabbit small intestine was suspended, a concentration of 1:100,000 and 1:200,000 caused a slight reduction in tone and a depression in the number of contractions per unit of time. Papaverine hydrochloride, 1:100,000, was much more effective in depressing the movements of the strip. Dilutions of 1:100,000 of the new compound had no effect on the isolated rabbit uterus in a quiescent stage, nor on the tissue contracted with epinephrine hydrochloride. The same concentration did not alter the normal movements of the guinea pig uterus, nor the spasm produced by extracts of the posterior lobe of the pituitary gland.

Unanesthetized Animals.—Motility of the small intestine of unanesthetized dogs was recorded by placing a balloon in previously constructed Thiry fistulas. Doses of 1.0 to 2.5 mg./Kg. by vein failed to bring about any appreciable change in the motility of the small intestine. In one instance 1.0 mg./Kg. caused vomiting.

Toxicity

Acute.—The acute toxicity of the phosphate of the new analog was determined by intravenous injection in 40 albino mice. The median lethal dose (LD_{50}) was 112.7 mg./Kg. Similar toxicity studies with papaverine hydrochloride showed the LD_{50} to be 33.1 mg./Kg. A summary of the toxicity data is given in Table I.

The oral toxicity was determined in albino rats by administration of aqueous suspensions of the hydrochloride and the phosphate of 6,7-dimethoxy-1-(4′-ethoxy-3′-methoxybenzyl)-3-methyl-isoquinoline, as well as papaverine hydrochloride. The median lethal dose (LD_{50}) for the hydrochloride was 1,961 mg./Kg.; and for the phosphate, 2,069 mg./Kg. Papaverine hydrochloride, by mouth, showed an LD_{50} of 745.6 mg./Kg. Thus, both the hydrochloride and the phosphate of the new compound are less toxic by oral and intravenous injection than papaverine hydrochloride.

Chronic.—For chronic toxicity studies the hydrochloride was employed. Four groups of five female albino rats each were fed diets containing 0.1, 0.2, 0.5, and 1.0% of the drug, respectively, for four weeks. The average gain in body weight and the food consumption declined slightly as the drug concentration in the diet was raised. However, even a daily average dose of 100 mg., over 50% of the LD_{50}, did not appreciably alter the growth curve.

All the animals survived the test period but one. This animal suffered an accidental death. At the end of four weeks the rats were sacrificed and submitted to necropsy. Gross and microscopic examination failed to reveal any pathologic changes attributable to the drug.

Addiction Liability

Analgesia.—Since papaverine is an alkaloid of opium, it was decided that the new derivative should be studied for the possible occurrence of analgesia and tolerance, although no evidence of addiction

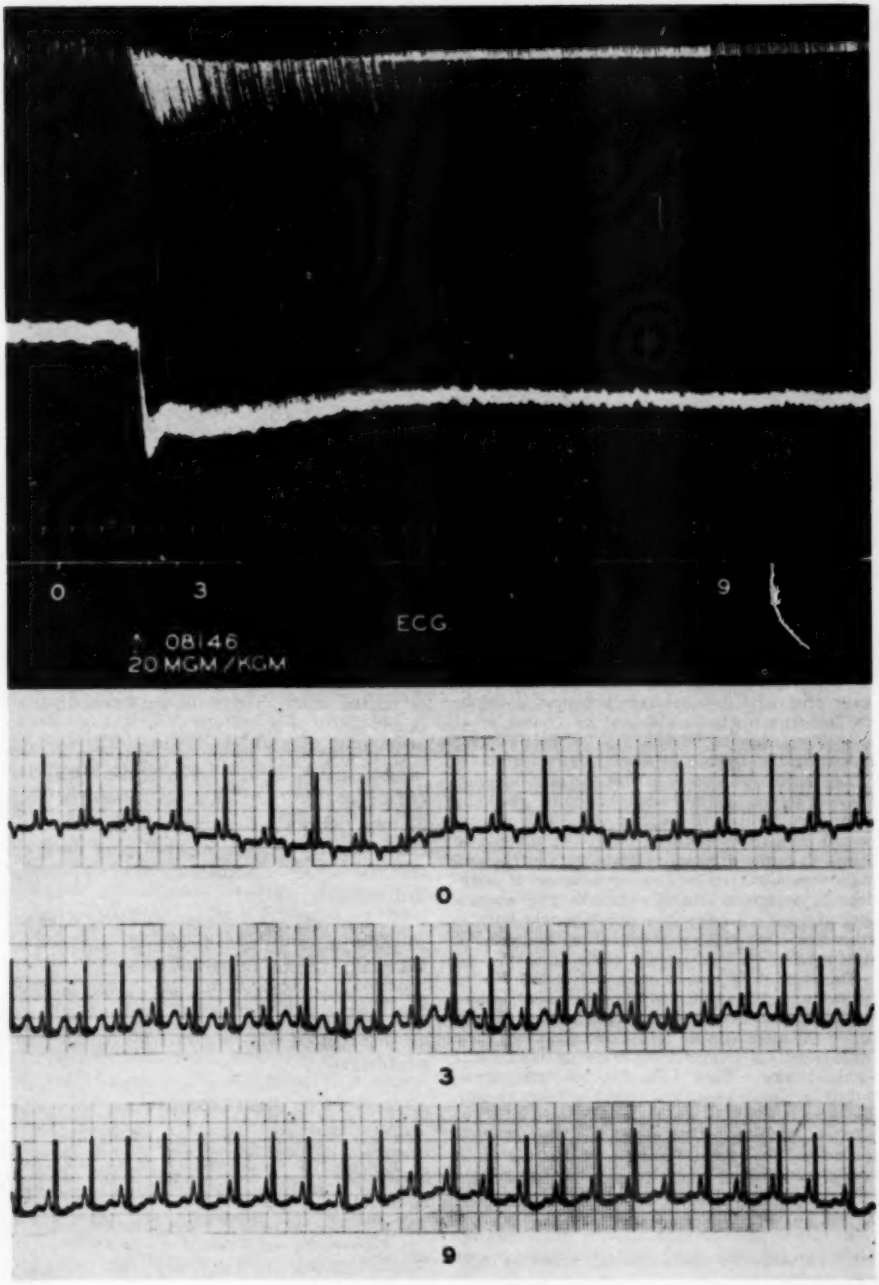

Fig. 1.—Blood pressure, respiration, and electrocardiograms.
The dog was anesthetized with phenobarbital sodium, and all injections were made by vein. From above downward, tracings are respiration, blood pressure, time in minutes, and signal. The numbers of the electrocardiograms correspond to the numbers in the kymogram.

TABLE I.—MEDIAN LETHAL DOSE (LD_{50}) IN RATS AND MICE FOR THE PHOSPHATE AND HYDROCHLORIDE OF 6,7-DIMETHOXY-1-(4′-ETHOXY-3′-METHOXYBENZYL)-3-METHYL-ISOQUINOLINE AND PAPAVERINE HYDROCHLORIDE

Drug	Animal	Route of Administration	Dose, Mg./Kg.	No. Died/-No. Used	LD_{50} ± S. E., Mg./Kg.
Phosphate	Mice	Intravenous	90	1/10	112.7 ± 4.1
			100	3/10	
			110	3/10	
			125	8/10	
Papaverine hydrochloride	Mice	Intravenous	30	3/10	33.13 ± 1.69
			33	6/10	
			36.5	7/10	
			40.0	6/10	
Phosphate	Rat	Oral	1,600	1/5	2,069 ± 387
			2,000	1/5	
			2,500	5/5	
Hydrochloride	Rat	Oral	1,600	0/5	1,961 ± 84
			2,000	3/5	
			2,500	5/5	
Papaverine hydrochloride	Rat	Oral	500	1/5	745.6 ± 62.6
			620	1/5	
			800	3/5	
			1,000	4/5	

liability has ever been proved with papaverine itself. Twenty-five mice were given intravenously 6,7-dimethoxy-1-(4′-ethoxy-3′-methoxybenzyl)-3-methyl-isoquinoline phosphate in a 0.5% solution. These animals were divided into five groups, each receiving a dose ranging from 12.5 to 100 mg. In none of the animals was there erection of the tail (Straub's sign) or loss of pain sensation, as tested by pinching of the tail. The highest dose, namely, 100 mg./Kg., killed two out of five mice.

Fifteen rats were injected intravenously with the same drug, and their reaction time was tested by the hot wire method elaborated by Davies, *et al.* (9). There was no increase in the reaction time indicating the absence of any analgesic action.

Tolerance.—The phosphate of the new isoquinoline derivative was administered to five dogs, by mouth, in the dose of 150 mg./Kg. This was repeated each day for fifteen days. At the end of fifteen days the drug was withdrawn and the animals were observed for any appearance of withdrawal symptoms. In all respects the animals appeared normal. One dog gained weight but the other four lost weight slightly during the period of treatment.

The same drug was incorporated in the diet to the extent of 1% and fed to 25 rats. This was continued for thirty-one days. At the end of this period the animals were fasted overnight and their acute toxicity determined the following day by oral administration. Their LD_{50} did not differ significantly as shown by a t test from the figure already presented in an above section under acute toxicity—1,710 ± 260 against 2,069 ± 387 mg./Kg. Thus it can be said that the new compound does not cause development of tolerance by repeated administration.

SUMMARY

1. In dogs by intra-arterial injection 6,7-dimethoxy-1-(4′-ethoxy-3′-methoxybenzyl)-3-methyl-isoquinoline as the hydrochloride or phosphate is equal to papaverine in dilating coronary and femoral arteries.

2. Doses of 10 and 20 mg./Kg. of the new compound as the phosphate by vein produces a fall in blood pressure. The larger dose causes an increase in respiratory rate in dogs.

3. Electrocardiograms show an increase in heart rate and a reversal of the T wave following 20 mg./Kg. of this compound by vein in dogs.

4. The synthetic analog has no significant effect on the isolated small intestine of the rabbit, or on the small intestine of the unanesthetized dog.

5. Intravenous injection of the new product in albino mice gives rise to the median lethal dose (LD_{50}) of 112.7 mg./Kg., as compared with 33.1 mg./Kg. for papaverine hydrochloride.

6. The new derivative, when given orally, is also less toxic to albino rats than papaverine hydrochloride.

7. Necropsy of albino rats following repeated administration of the hydrochloride disclosed no lesions attributable to the compound.

8. The new papaverine analog shows no analgesic action in mice and rats, and no tolerance development in dogs and rats by repeated administration.

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Notes on the Determination of Theophylline, Alone and in Combinations, by the U. S. P. Aminophylline Method*

By GEORGE B. GRIFFENHAGEN† and EDWARD S. BRADY‡

In adapting argentometric methods to the assay of certain nitrogenous organic compounds, the U. S. P. assay of theophylline was reviewed in detail. Increased precision of results was noted when (*a*) the precipitated mixture was cooled before filtration, and (*b*) when the ammonia presently required in the assay was omitted. The substitution of other buffers (lactates and acetates) for the ammonia was not advantageous. Physical and chemical characteristics of silver theophyllinate were reviewed, and modification of the present U. S. P. assays of theophylline and aminophylline is suggested.

THE PRESENT U. S. P. method for the assay of theophylline in aminophylline became official with the Eleventh Revision, Second Supplement (1), and has continued virtually unchanged through subsequent pharmacopeial revisions. It has since been adopted for the determination of theophylline in theophylline tablets, theophylline and sodium acetate, and theophylline and sodium acetate tablets. While other official works and standard methods (2, 3) employ gravimetric procedures, the U. S. P. assay is argentometric, and is based upon the reaction of theophylline with 0.1 *N* silver nitrate to form silver theophyllinate, $Ag(C_7H_7N_4O_2)$. Precipitation is carried out in a slightly ammoniacal solution (about 1 per cent); the mixture is digested for fifteen minutes on a water bath and filtered while hot. On cooling, excess 0.1 *N* silver nitrate is titrated with 0.1 *N* ammonium thiocyanate using ferric ammonium sulfate T. S. as an indicator (4).

Since silver nitrate forms insoluble compounds with many nitrogenous organic substances, assay methods similar to the official procedure for theophylline in its compounds have been suggested for many such substances (5–7). These methods differ from the U. S. P. in the details of temperature of filtration, quantity of ammonia used, and addition of buffer salts. In recent re-evaluations of these methods (8), it has been noted that many of the silver precipitates fail to form, or are redissolved, when a slight excess of ammonia is present. Further, some partially dissolve upon moderate heating, and precipitation has been observed in theophylline filtrates upon cooling after warm filtration. Also, the use of buffers in theophylline determinations has not been reported. These facts have made an investigation of the present U. S. P. method for the determination of theophylline in compounds seem warranted.

EXPERIMENTAL

Samples of theophylline and aminophylline were assayed by the U. S. P XIII method for theophylline in aminophylline, as shown in Table I. Samples were then analyzed by modifying this method by allowing the sample to cool to room temperature before filtration. Results are given in Table I under modification I. The addition of 2 cc. of a 10% solution of sodium acetate as a buffer prior to precipitation yielded the results given in Table I as modification II, and with 2 cc. of a 50% solution of sodium *r*-lactate as a buffer, those of modification III. Samples were then analyzed by omitting the ammonia required by the U. S. P., using no buffer, and cooling the precipitated solution to room temperature before filtration. Results are given in the table under modification IV. This last modification appears to give the most uniform results.

DISCUSSION

When the U. S. P. method was used, precipitation sometimes occurred in the hot filtrate as it was cooled to room temperature for titration. Many other organic silver complexes are quite soluble in hot water (8). Other similar argentometric methods direct cooling before filtration (5, 9). By comparison of the results in the table, it may be seen that the maximum deviation of results from the average values obtained appreciably decreases if the mixture is not filtered "while still warm" (4).

Mangouri and Milad (5) suggested the use of a sodium acetate buffer in the argentometric assay of barbituric acid derivatives. They called attention to the fact that such solutions tended to become more acidic as the silver compounds were precipitated, and that this condition sometimes caused incomplete precipitation, and could be prevented by the use of the buffer. However, when sodium acetate was used in the theophylline determinations, unusually high percentages were obtained (modification II). Due to the possibility that this error was caused by the co-precipitation of silver acetate, a salt which would yield a more soluble silver compound, sodium *r*-lactate, was employed in a further series of deter-

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Presented to the Scientific Section, A. PH. A., Atlantic City meeting, May 1950.
† Fellow of the American Foundation for Pharmaceutical Education, University of Southern California, Los Angeles.
‡ Associate Professor of Pharmacy, University of Southern California, Los Angeles.

minations. The high values again obtained (modification III) and the formation of bulky, gelatinous precipitates which were difficult to filter and wash, and which were relatively unstable, seemed to indicate that addition of alkaline buffers would not increase the accuracy of the U. S. P. method.

The use of ammonia in the argentometric precipitation of the xanthines was first suggested by Kunze (10) for the precipitation of theobromine. No other supporting evidence for the use of ammonia has been found in the literature. Ammonia was used in the proposed U. S. P. XI assay for theophylline in aminophylline (11) and was required in the adopted U. S. P. XI assay, although the amount was changed from 7 to 8 cc. per sample. While ammonia may aid the initial solution of the sample, no difficulty was encountered when it was omitted. The character of the precipitate is relatively unchanged as to stability and ease of filtration in non-ammoniacal solution.

On the other hand, silver theophyllinate and most other silver precipitates are soluble in ammonia, and this places a quantitative aspect upon the amount of ammonia used which the assay does not closely control. Its omission, accompanied by cold filtration, yielded the best series of determinations (modification IV).

It should be noted that the results for theophylline in aminophylline when ammonia is omitted are somewhat higher than those obtained when it is used. The reason for this is not apparent, but it is not due to any reaction of the ethylene diamine, as was shown by running blank determinations with ethylene diamine alone.

It was also observed that the precipitates formed were less gelatinous when a larger excess of silver nitrate was present, and the use of 50 ml. of 0.1 *N* silver nitrate in place of 20 ml. aids in forming a less bulky precipitate.

TABLE I.—DETERMINATION OF THEOPHYLLINE ALONE AND IN AMINOPHYLLINE BY U. S. P. METHOD AND FOUR MODIFICATIONS

Method	Sample	Sample Wt.	Theophylline, %
U. S. P. XIII	Theophylline A	0.3231	99.08[a]
	Theophylline B	0.2966	98.31
	Theophylline C	0.3275	97.86
	Theophylline D	0.2794	98.57
	Av.		98.45
	Aminophylline A	0.2349	80.01[b]
	Aminophylline B	0.3470	81.48
	Aminophylline C	0.2167	81.09
	Av.		80.86
Modification I[c]	Theophylline A	0.3088	98.41[a]
	Theophylline B	0.3335	98.38
	Theophylline C	0.3126	98.12
	Theophylline D	0.2579	98.38
	Av.		98.32
	Aminophylline A	0.2026	80.67[b]
	Aminophylline B	0.2171	81.34
	Aminophylline C	0.1717	80.98
	Av.		81.00
Modification II[d]	Theophylline A	0.2597	101.77[a]
	Theophylline B	0.2525	103.02
	Theophylline C	0.2476	103.63
	Theophylline D	0.2503	99.58
	Av.		102.00
	Aminophylline A	0.3160	84.53[b]
	Aminophylline B	0.4000	83.45
	Aminophylline C	0.3720	83.69
	Av.		83.89
Modification III[e]	Theophylline A	0.2500	97.94[a]
	Theophylline B	0.2544	102.37
	Theophylline C	0.2530	100.65
	Theophylline D	0.2503	97.61
	Av.		99.14
	Aminophylline A	0.2450	87.80[b]
	Aminophylline B	0.2520	86.90
	Aminophylline C	0.2430	88.92
	Av.		87.87
Modification IV[f]	Theophylline A	0.1585	98.04[a]
	Theophylline B	0.2013	98.07
	Theophylline C	0.2490	97.93
	Theophylline D	0.3470	98.12
	Theophylline E	0.2012	97.99
	Theophylline F	0.2015	97.84
	Av.		98.00
	Aminophylline A	0.1448	83.50[b]
	Aminophylline B	0.1731	82.86
	Aminophylline C	0.2930	82.15
	Aminophylline D	0.2739	83.30
	Aminophylline E	0.3181	83.35
	Aminophylline F	0.1920	82.94
	Av.		83.02

[a] Expressed as theophylline monohydrate.
[b] Expressed as anhydrous theophylline.
[c] Precipitated mixture cooled before filtration.
[d] 2 cc. of 10% sodium acetate added before precipitation.
[e] 2 cc. of 50% sodium *r*-lactate added before precipitation.
[f] Ammonia omitted and mixture cooled.

CONCLUSIONS

1. The U. S. P. XIII method for the determination of theophylline in aminophylline has been subjected to laboratory evaluation in original and modified procedures.

2. The cooling of the precipitated mixture before filtration appeared to increase the precision of the assay.

3. The addition of sodium acetate or sodium *r* lactate as a buffer was not advantageous.

4. The addition of 8 cc. of ammonia T. S. as directed in the present monograph appears unnecessary and may constitute a source of error.

5. Increasing the volume of 0.1 *N* silver nitrate to 50 ml. per sample aids in forming a less bulky precipitate.

6. It is suggested that the present assay may be improved by omitting the ammonia T. S., increasing the volume of 0.1 *N* silver nitrate used, and cooling the precipitated mixture before filtration.

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Measurement of Gravity Filtration*

By RUTH MOOTE KROEGER and H. GEORGE DeKAY

A survey of the literature reveals the need for a standardized procedure for testing the speed of filter papers. A practical electrical apparatus is described for testing the speed of gravity filtration. Preliminary tests on 44 brands of filter papers show discrepancies in the observed rate of filtration using this method and the commercial reports for rapidity.

FILTRATION is one of the most common and important operations of pharmacy, finding application in both commercial manufacturing of medicinals and compounding of prescriptions. It may be defined as the process of separating undissolved solid or semisolid material from a liquid by passing the mixture through a porous material called the filter medium, which allows the clarified liquid to pass through and retains the undissolved solid particles. There are two general types of filtration, pressure filtration and gravity filtration. Pressure filtration is seldom used by the compounding pharmacist since it involves the use of expensive equipment to establish abnormal conditions of pressure or vacuum, and is not practical for small volumes. The compounding pharmacist most commonly uses gravity filtration, a process consisting of using a funnel, funnel support, and an unspecified type of filter paper folded according to the individual's preference. It can be readily seen that the speed of filtration of any liquid would vary according to the type of filter paper used. Filter paper manufacturers have classified their papers as "slow," "medium," and "fast" filtering, but this classification is unreliable since these rates are not based upon any one standard method of determination. The pharmacist would be greatly helped if he could use filter papers compared by a standard method which would indicate the type of filter paper best suited to his needs.

Most of the experimental work on filtration in the past few decades has dealt with pressure filtration because of its importance to industry. The literature reveals that prior to 1917 most of the reports on gravity filtration concerned the types of filter paper, their manufacture, uses, and methods of folding. In 1917 Herzberg (1) reported an apparatus for testing filtration rate of unfolded filter paper under a 5-cm. head of water. In 1922, Griffin and Parish (2) reported an apparatus for testing unfolded filter paper and determined penetrability figures which varied inversely with the speed of the paper. In 1942 Bogaty and Carson (3) devised a method using folded filter paper and ran tests to develop a water-filtration coefficient. Scribner and Wilson (4) in 1945 used the Bogaty and Carson method in the evaluation of analytical filter papers. A manufacturer's booklet (5) issued in 1945 reports another method for testing filter papers in the flat position. A careful study of these procedures shows that there is a need for a more concrete basis upon which to classify filter papers as to their rate of filtration. Some of the methods are abnormal pharmaceutically because the filter paper is not folded, while others, although folded paper is used, are not duplicating the physical conditions and phases of gravity filtration as it is employed by the compounding pharmacist.

The purpose of this work is to attempt to develop a satisfactory method for the determination of the rate of filtration through filter paper in the folded state solely by means of gravity.

EXPERIMENTAL

Apparatus.—The apparatus used for the most satisfactory method consisted of a delivery vessel, a standard funnel, a receiving vessel with electrical wiring, and a "Time-It" recorder.

Delivery Vessel.—A 250-cc. Pyrex Erlenmeyer flask was modified so that it had a bent 10-mm. Pyrex tube joined at the side near the bottom of the flask, extending upward 7 cm. above the flask opening, and about 0.5 cm. away from the edge of the neck of the flask. A rubber stopper containing a 2-mm. Pyrex glass stopcock was fitted into the flask and the air inlet tube was then cut off 1.5 cm. shorter than the stopcock tube ending. This delivery vessel was calibrated at the 150-cc. volume level.

Funnel.—The funnels used were ordinary 60-degree angle funnels.

Receiving Vessel.—The receiving vessel was constructed by placing a 34-cm. length of 12-mm. diameter Pyrex glass tubing inside a 35-cm. long Pyrex tube of 25-mm. diameter, and sealing these tubes together at the upper end. The lower end of the 25-mm. cylinder was constricted, and a glass stopcock was attached at this point. Platinum wire leads were sealed into the outer tube at various heights and were connected to posts of a three-way switch. The common lead of the switch was connected to one of the high voltage leads of an ordinary 8-v. bell transformer mounted in a box

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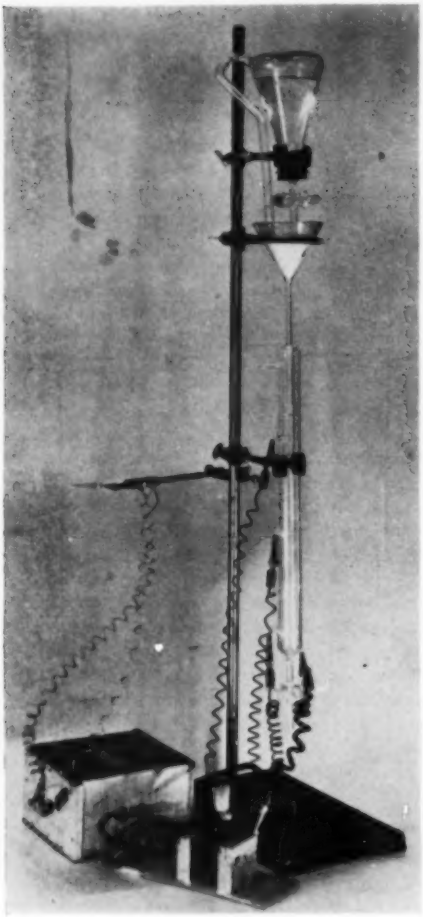

Fig. 1.—Apparatus.

as described by Haley and Edwards (6). One wire from a 110-v. outlet was connected to the common lead at the base of the cylinder and the other wire from the 110-v. outlet led to the transformer. A signal magnet, used as a sound device, was attached at the low voltage side of the transformer.

This apparatus is illustrated by Fig. 1 and the wiring diagram in Fig. 2.

Method.—The standard funnel containing 11 cm. plain folded filter paper, wetted with 25 cc. of water and the excess water drained off, was placed in a stationary support with the stem parallel to and touching the side of the inner tube of the receiving vessel. Sodium chloride, crystalline, 0.3 Gm., to conduct the electrical current, was introduced into the receiving vessel. The delivery vessel was filled to the calibrated mark with the solution to be tested, the stopper inserted, and the vessel inverted into a stationary position above the funnel. The timer was started when the stopcock of the delivery flask was opened, and when contact was made between the common lead at the base of the cylinder and the volume lead, the circuit was completed, causing a buzz on the signal magnet, and the time was read from the "Time-It" recorder.

The instrument was calibrated by introducing distilled water containing 0.2% sodium chloride dropwise directly into the receiving vessel from a burette. The volume was read when the drop made contact with each lead, as evidenced by the buzzing of the signal magnet. The average reading for each volume lead established them to be at 11.6 cc., 59.3 cc., and 107.8 cc.

DISCUSSION

This method was desirable in that the normal filtration procedure was maintained by the use of folded and wetted filter paper, the delivery flask could be placed into position, and the delivery of the liquid was not started until the stopcock was opened, a regulated flow of the liquid ensued, the level of the liquid in the funnel was maintained constant, and the operator could simultaneously start the time recorder and delivery flow. Tests of various filter paper filtration rate can be made using different solutions. These results can then be compared with standard values using distilled water as the test solution. This would give filtration speed ratios for various filter papers and solutions in order to determine the best filter paper to be used. The testing of this method on 44 different filter papers of different manufacturers has shown that discrepancies do exist between the actual filtration rates obtained and those claimed by the manufacturers or/and the distributors of the filter papers.

SUMMARY AND CONCLUSIONS

1. A survey of the literature reveals the definite need for a practical standardized method for evaluating filter papers used in pharmaceutical gravity filtration. The methods previously stud-

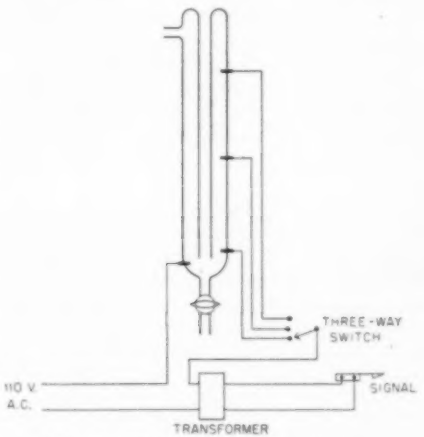

Fig. 2.—Wiring diagram of apparatus.

ied do not duplicate the normal usage of folded and wetted papers in conjunction with a funnel.

2. A new apparatus and method has been developed for determining filtration speed of filter papers. This method approximates the normal filtration process and appears satisfactory since any error factors can be standardized and leaves the filter papers as the only variable factor affecting the filtration time.

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Preparation and Toxicity of Some New Organic Gold Compounds*,†

By J. LEON LICHTIN‡ and LOYD E. HARRIS§

Methods of preparation are described for five organic gold derivatives, in four of which the gold is attached to the balance of the molecule through the element nitrogen. Preliminary toxicity tests on three of them are included.

GOLD COMPOUNDS are used therapeutically in the treatment of rheumatoid arthritis (1). The large number of clinical investigations which have evaluated this form of therapy have shown that about one-half of the patients obtain significant relief.

Gold compounds, although offering therapeutic effectiveness, have the disadvantage of causing frequent toxic reactions. Because of this, there have been many attempts to produce new gold compounds which would be therapeutically effective, but less toxic.

There are three compounds which have extensive therapeutic use in this country. In all of these compounds, the gold is linked to the balance of the molecule through the element sulfur. It appeared worth while to investigate some new gold compounds in which nitrogen is the element which links the metal to the rest of the molecule.

In this investigation an attempt has been made to prepare several gold derivatives of organic nitrogen bases, so selected that their gold derivatives could possibly be useful in therapeutics. The chemical structures of the new gold derivatives have been studied and structural formulas have been postulated for each. These are based on those of Gibson (2) and his collaborators. As a first step toward a clinical trial on human patients, these gold compounds have been subjected to toxicity tests on mice.

The gold chloride used throughout the investigation was either of two forms marketed by Merck and Company, namely, the reagent grade, or the acid yellow form. The formula of both compounds was assumed to be $HAuCl_4 \cdot 4H_2O$ (412) and is termed chlorauric acid.

EXPERIMENTAL

In the developmental preliminary experiments, the variables chiefly manipulated were pH, the ratio and the dilution of the reactants. The precipitated gold compounds were observed for stability, elegance, crystalline form, and yield. The filtrates from these precipitates were observed for stability and the development of a secondary precipitate. Having determined an optimum preparatory procedure, it was possible to produce high yields of fine-looking crystalline precipitates, which required no further purification other than washing with water, and clear filtrates which underwent no further change.

Preparation of Gold Cinchophen.—A filtered solution containing 3.296 Gm. (8×10^{-3} *M*) of chlorauric acid, and 0.5 Gm. of sodium chloride in 330 ml. of water was placed into a 1,000-ml. Erlenmeyer flask. To this was added 40 ml. of 37–38% hydrochloric acid, 3.984 Gm. (16×10^{-3} *M*) of cinchophen, and a few boiling chips. The mixture was refluxed for seventy-five minutes, during which time a crystalline precipitate was formed. The

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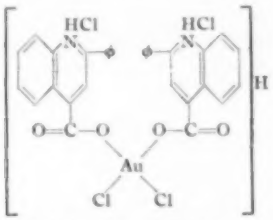
‡ Fellow of the American Foundation for Pharmaceutical Education. At present, member of the Department of Pharmacy, Cincinnati College of Pharmacy.
§ Professor of Pharmaceutical Chemistry, College of Pharmacy, Ohio State University, Columbus, Ohio.

mixture was cooled and permitted to stand overnight. The crystals were then filtered with suction, washed with cold water, and dried. The filtrate from the crystals was pale yellow-green, and not intensely colored. It was placed in the refrigerator for three months, after which time a small amount of crystals had precipitated and the liquid was practically colorless. The yield of gold cinchophen, including 0.075 Gm. obtained from the filtrate, was 6.527 Gm. (97% of theory). The substance melted with decomposition at 228–231°.

In a series of six experiments, it was determined that in the formation of gold cinchophen, two molecules of cinchophen combined with one molecule of chlorauric acid.

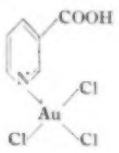
The formula of gold cinchophen is postulated to be:



Anal.—Calcd. for above formula: Au, 23.6; N, 3.34; Cl, 16.9. Found: Au, 24.0; N, 3.39; Cl, 16.3.

Preparation of Gold Nicotinic Acid.—A solution containing 2.46 Gm. (2×10^{-2} *M*) of nicotinic acid in 246 ml. of water was placed into a separatory funnel. This was added dropwise to a mechanically stirred and filtered solution of 8.24 Gm. (2×10^{-2} *M*) of chlorauric acid and 1.2 Gm. of sodium chloride in 824 ml. of water, contained in a 2-L. beaker. The addition required three minutes. The precipitate, which formed in a few minutes, was stirred for about ten minutes longer. After the short period of stirring, the precipitate was filtered off, washed with water, and dried. The yield of gold nicotinic acid, which included 0.700 Gm. obtained from the filtrate, was 7.747 Gm. (91% of theory). The substance melted with decomposition at 267–269°.

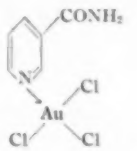
The formula of gold nicotinic acid is postulated to be:



Anal.—Calcd. for above formula: Au, 46.3; N, 3.28; Cl, 25.0. Found: Au, 46.4; N, 3.30; Cl, 24.9.

Preparation of Gold Nicotinamide.—A solution containing 3.66 Gm. (3.0×10^{-2} *M*) of nicotinamide in 370 ml. of water, was placed into a separatory funnel. This was added dropwise, but fairly rapidly, to a mechanically stirred and filtered solution of 6.18 Gm. (1.5×10^{-2} *M*) of chlorauric acid and 0.9 Gm. of sodium chloride in 2,490 ml. of water contained in a 4-L. beaker. Precipitation began within a few minutes, after which the mixture was stirred for eighteen minutes. The precipitate was removed by filtration, washed with water, and dried. The filtrate was only feebly colored; after three months' storage in a refrigerator, it did not deposit any more crystals. The yield of gold nicotinamide was 5.908 Gm. (92% of theory). The substance melted with decomposition at 243–245°.

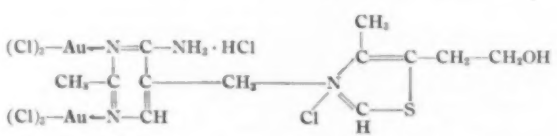
The formula of gold nicotinamide is postulated to be:



Anal.—Calcd. for above formula: Au, 46.4; N, 6.58; Cl, 25.0. Found: Au, 46.8; N, 6.64; Cl, 28.4.

Preparation of Gold Thiamine.—A filtered solution containing 824 mg. (2×10^{-2} *M*) of chlorauric acid and a few crystals of sodium chloride in 82 ml. of water was placed into a 250-ml. beaker. To this was added 4 ml. of 10% hydrochloric acid. To the mechanically stirred gold solution was added, all at once, a solution of 674 mg. (2×10^{-3} *M*) of thiamine chloride in 13.5 ml. of water. The precipitate, which formed almost immediately, was stirred for twenty to twenty-five minutes. The *p*H of the reaction mixture was 1.0 or less. The precipitate was filtered off with suction and dried in air. No significant quantity of crystals was obtainable from the filtrate. The yield of gold thiamine was 865 mg. (92% of theory). The substance decomposed between 157–167°.

The formula of gold thiamine is postulated to be:

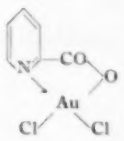


Anal.—Calcd. for above formula: Au, 41.74; N, 5.93; Cl, 30.1. Found: Au, 41.37; N, 5.81; Cl, 28.95.

Preparation of Gold Picolinic Acid.—A filtered solution containing 824 mg. (2×10^{-3} *M*) of chlorauric acid and a few crystals of sodium chloride in 82 ml. of water was placed into a 250-ml. beaker. This solution was then adjusted to *p*H 6.5 by the addition of sufficient sodium bicarbonate. To the mechanically stirred gold solution was added, in a dropwise manner, a solution of 542 mg. (4.4×10^{-3} *M*) of picolinic acid in 50 ml. of water. The reaction mixture was stirred for five minutes, and the pre-

cipitate was promptly filtered off, washed with water, and dried. No significant quantity of crystals was obtained from the filtrate. The yield of gold picolinic acid was 520 mg. (67% of theory). The substance melted with decomposition at 190–191°.

The formula of this substance is postulated to be:



Anal.—Calcd. for above formula: Au, 50.5; N, 3.59; Cl, 18.2 Found: Au, 50.9; N, 3.53; Cl, 18.35.

General Properties of the Gold Compounds.—This group of gold compounds are all crystalline, and rather elegant-appearing bright yellow solids. With the exception of gold thiamine, the compounds all melt with decomposition at a fairly sharp temperature range. They are all insoluble in water, but possess varying degrees of solubility in organic solvents. Gold cinchophen and gold nicotinic acid form solutions in dilute sodium bicarbonate solutions which can be neutralized without causing immediate precipitation. All of the compounds are stable in air, light, and water. A suspension of them in water, however, is readily decomposed by sulfur dioxide solution.

Preliminary Toxicity Tests.—It was not possible to prepare dosage forms, suitable for parenteral injection, for gold nicotinamide and gold picolinic acid. Suitable dosage forms, however, were prepared from gold thiamine, gold cinchophen, and gold nicotinic acid. The last two compounds were dissolved in dilute sodium bicarbonate solution. These solutions were then neutralized with the necessary amount of glacial acetic acid.

The technique used in these tests was as follows: healthy Swiss female mice weighing between 20 and 30 Gm. were used. The solutions were injected with moderate speed into the tail veins using tuberculin syringes and a 25-gauge, $^3/_4$-in. long needle. All doses administered were based on the dose per 20-Gm. mouse, and doses for mice weighing other than 20 Gm. were adjusted accordingly. In both the preliminary range-finding tests, and in the toxicity tests themselves, all mice that died within the two-week period following the injection were counted. However, all the mice, that succumbed, died within two days. This is in direct contrast to the toxicity of gold sodium thiosulfate, which was observed to develop quite slowly.

Toxicity of Gold Thiamine.—Doses of only 0.5 mg. per mouse were immediately fatal. Because of the smallness of this dose, the exact toxicity of this compound was not determined, since it was not necessary to do so for the purposes of this investigation.

Toxicity of Gold Nicotinic Acid.—Both the LD_{50} and LD_{100} were determined to lie in the very narrow range of 1.70 and 2.40 mg. equivalent to 0.79 and to 1.12 mg. of gold. Forty mice were used in this determination.

Toxicity of Gold Cinchophen.—Preliminary range-finding tests in which only a few animals were used showed that the LD_{50} dose was probably between 4 and 6 mg. In the larger scale tests in which 48 mice divided among five dose levels were used, the LD_{50}, as determined both by the integration method of Behrens and the graphical method of Miller and Tainter, was 5.70 mg. of gold cinchophen, which is equivalent to 1.37 mg. of gold.

The weight of gold in gold cinchophen required to produce the LD_{50} was about 1.7 times that of the gold contained in the average of two LD_{50} determinations (in which 37 and 54 mice were used) made on gold sodium thiosulfate by the authors during some preliminary experiments.

SUMMARY AND CONCLUSIONS

1. Five new organic gold compounds have been prepared. The gold is attached to the organic molecule through the element nitrogen in four of them, but through the carboxyl groups in the fifth (gold cinchophen).

2. These new compounds are easily prepared in high yield, and in crystalline form. The substances are stable in air, light, and water.

3. The determined structural formulas corroborate the conclusion of Gibson that tervalent gold is 4-covalent.

4. Chelated structures are not formed when gold derivatives of nicotinic acid (a β-carboxylic acid), or nicotinamide, are produced. The gold derivative of picolinic acid (an α-carboxylic acid), however, was shown to possess a chelated structure.

5. The acute toxicity of two of the new gold compounds has been determined.

It is concluded that organic gold compounds, in which the gold is linked through nitrogen to the organic molecule, deserve further study. Perhaps a gold compound of this class could be prepared which would be even less toxic and would be sufficiently promising to warrant a clinical trial.

REFERENCES

(1) Hollander, J. L., "Comroe's Arthritis," Lea & Febiger, Philadelphia, 1949, pp. 258–293.

(2) Gibson, C. S., "Recent Investigations in the Chemistry of Gold," *Brit. Assoc. Advancement Sci. Rept.*, **1938**, pp. 35–56.

Note

A Note on a Procedure for the Removal of Coating from Coated Tablets*

By FRANK V. HERREYGERS

REMOVING the coating from tablets considered unsuitable for release by control laboratories is frequently necessary. Washing such tablets with water and removing the water by alcohol is the classical, but not very satisfactory, procedure. Recently Curlin (1) published an improved method using isopropyl alcohol of increasing strength to remove the coating. Unfortunately this method requires an especially modified polishing head, which is not always at hand.

During the course of the development of an improved coating procedure, we were often faced with the need to remove coatings from tablets. Since we had no polishing head at hand, we sought a more expedient and less expensive procedure which would avoid both the use of alcohol and the use of a modified polishing head. As a result of our experience we can recommend the following method.

Take a nearly equal weight of rejected coated tablets of a size different from those to be washed, and selected so that a simple screening will separate the two. Rotate these rejected tablets in a heated coating pan. Soak the tablets, from which the coating is to be separated, in water until the coating is removed to the desired extent. Drain off the water, transfer the washed tablets into the coating pan, and allow the tablets to roll until dry. The heated rejected tablets will absorb the residual water from the washed tablets. Separate the dried tablets by screening and continue the drying in a drying cabinet.

The rejected tablets, after having been thoroughly dried, can be used again for other washing operations.

The procedure gave very good results. The coating was evenly removed and the washed tablets were very smooth. The re-coating process can be started immediately, and thus loss of time is avoided.

* Received November 21, 1950, from the private laboratory of Frank V. Herreygers, 88 Steenebrug, Borgerhout-Antwerp, Belgium.

REFERENCE

(1) Curlin, L. C., THIS JOURNAL, **39**, 112(1950).

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BOOKS RECEIVED

The Polarographic Method of Analysis. By OTTO H. MÜLLER. Chemical Education Publishing Co., Easton, Pa., 1951. xii + 209 pp. Illustrated. 13.5 x 21 cm. Price $3.50.

Synthetische Methoden der Organischen Chemie. Vol. 4. By W. THEILHEIMER. Interscience Publishers, Inc., New York, 1950. xi + 360 pp. 16 x 23.5 cm. Price $9.25.

Perfumery Synthetics and Isolates. By PAUL Z. BEDOUKIAN. D. Van Nostrand Company, Inc., New York, 1951. viii + 488 pp. 15.5 x 24 cm. Price $7.

The Analytical Balance—Its Care and Use. By WILLIAM MARSHALL MACNEVIN. Handbook Publishers, Inc., Sandusky, Ohio, 1951. xiii + 60 pp. 13.5 x 20.5 cm. Price $1.50.

Annual Report on Essential Oils, Aromatic Chemicals, and Related Materials. 1947–1948. Schimmel and Co., Inc., New York, 1951. 125 pp. Illustrated. 15 x 23 cm. Price in U. S. A. and Canada, $3.; elsewhere, $3.25.

Federal Food, Drug and Cosmetic Law Administrative Reports, 1907–1949. Commerce Clearing House, Inc., Chicago, 1951. xvi + 1,446 pp. 17 x 25 cm. Price $22.50.

Adsorption and Chromatography. By HAROLD GOMES CASSIDY. Interscience Publishers, Inc., New York, 1951. xix + 360 pp. Illustrated. 15 x 23.5 cm. Price $7.

Amino Acids and Proteins. By DAVID M. GREENBERG. Charles C Thomas, Springfield, Ill., 1951. ix + 950 pp. Illustrated. 16 x 26 cm. Price $15.

Austria-Codex 1950. 6th ed. By OTTO ZEKERT, WOLFDIETRICH WEIS, and HANS BRAUNER. Österreichischer Apotheker-Verlag, Vienna, 1950. 600 pp. 10.5 x 15 cm.

Salben, Puder, Externa (Die äusseren Heilmittel der Medizin). 3rd ed. By HERMANN V. CZETSCH-LINDENWALD and FRIEDRICH SCHMIDT-LA BAUME. Springer-Verlag, Berlin, 1950. xi + 492 pp Illustrated. 16 x 23.8 cm. Price DM 36.

Book Reviews

The Analytical Balance—Its Care and Use. By WILLIAM MARSHALL MACNEVIN. Handbook Publishers, Inc., Sandusky, Ohio, 1951. xiii + 60 pp. 13.5 x 20.5 cm. Price $1.50.

This little book will be useful to every laboratory and to every science student. In simple, direct language it provides practical information about the care and the use of the analytical balance. It discusses the parts of the balance, the procedures used for making accurate weighings and for testing the balance for errors. Instructions are given for detecting the need for adjustment of the balance, and the adjustments that can be made by the chemist are described. The reader is referred to expert balance repair men when the more difficult corrections and adjustments are needed.

Selected methods are given for the calibration of weights, and, fortunately, the calibration of the chain and the bar-type rider of the more modern balance is included.

There is also a brief historical review of balance manufacturing and a list of manufacturers of analytical balances used in American laboratories. Especially valuable is the list of qualified balance repair specialists.

The selection of methods for calibrating weights, for weighing, and for cleaning the balance are very good. It is fortunate that the author has refrained from going into great detail on the theory of weighing and the physics underlying the modern analytical balance, for thus he has preserved its usefulness as a handbook for frequent reference.

There are a few places where additions might be made to improve the value of the book. For example, there might be prescribed the optimum sensitivity for given types of weighing, and directions given for adjustment of the pointer swing to achieve this. Further, it would be useful to have a method for testing a projection beam balance, and perhaps the newer Gram-matic and keyboard types. A little more detail about the use of magnetic dampers would also be helpful.

This book is recommended to all who use the analytical balance, and as a text for student instruction.

Distillation Equilibrium Data. By JU CHIN CHU. Reinhold Publishing Corporation, New York, 1950. v + 304 pp. 15.5 x 23.5 cm. Price $6.

This book collects, for the first time since 1940, the available vapor-liquid equilibrium data, containing all such data published up to the end of 1949. All published data were included unless some reasons existed for belief of its inferiority. In some cases the information contained in the original publications had to be converted or recalculated in order that the data contained in this book be uniform.

The systems are divided into the large groups binary, ternary, and multiple-component systems, and individual systems are listed alphabetically according to the common name of one of the components. This arrangement is supplemented by the use of system numbers and a complete index which lists components by formula as well as name; thus one has no difficulty in finding the system in which he is interested. References to the original literature are always included. There are a total of 176 systems in the book.

This compilation should prove to be a convenient, time-saving, and dependable reference for the chemical engineer or other worker who is engaged in design or operation of distillation or extraction apparatus.

Laboratory Manual of Elementary Organic Chemistry. 2nd ed. By GEORGE HOLMES RICHTER. John Wiley and Sons, Inc., New York, 1951. ix + 146 pp. 15.5 x 23.5 cm. Price $1.80.

This manual is especially designed to appeal to the interest of students of the biological sciences, and it seems unusually well suited for students of pharmacy. The experiments include the usual qualitative tests for elements. Illustrations of melting point determination, fractional distillation, steam distillation, etc., and also the common name reactions that are usually given in elementary organic laboratory, such as the Würtz reaction, synthesis of ether, Haloform reaction, Cannizzaro reaction, and Friedel-Crafts reaction are included.

Of special interest is the fact that a number of experiments call for synthesis or isolation of medicinal or biochemical products. Hexamethylene tetramine, *dl*-Valine, methyl salicylate, D.D.T., Veronal, acetanilide, phenacetin, Anesthesine, aspirin, chloramine-T, guaiacol, Atophan, caffeine, urea, and eugenol are examples.

The laboratory directions are simple and sufficiently detailed so that the student will find them easy to understand and follow. There are some questions at the end of the experiments, though these are few in number, and the instructor will undoubtedly wish to supplement these with questions of his own.

The instructor will find the list of materials required of special value in preparing for the laboratory as well as for ordering.

This manual deserves examination by every teacher of elementary organic chemistry, and especially by those who teach pharmacy or premedical students.

Ion Exchange Resins. By ROBERT KUNIN and ROBERT J. MYERS. John Wiley and Sons, Inc., New York, 1950. xi + 212 pp. Illustrated. 15 x 23.8 cm. Price $4.75.

This book is a welcome addition to the chemical literature. It presents for the first time in book form a survey of the ion exchange resins and their applications.

In about the detail that one would expect in a special topics course, this book presents the theory and mechanism of ion exchange, followed by separate

discussions of anion and cation exchange resins and their functions. Of particular value is the notation made by the authors as to which type of mathematical treatment can be used for a given type of resin, and under what conditions. Practical methods for measuring exchange rates and capacities are described, and factors influencing rates are discussed. The use of data obtained by experimental columns is well illustrated by charts and tables.

The use of ion exchangers for deionization of water is described in some detail, and diagrams of typical setups are given. Other applications are also described and illustrated. Of special interest is the description of various columns to be used in evaluating resins. The book seems to be adequately referenced throughout.

This book will serve well as an introduction to the use and evaluation of ion exchange resins, and should be of considerable value in aiding one to select a resin for a particular job. It should be worth-while reading for the student as well as the industrial chemist.

Industrial Research Laboratories of the United States (Including Consulting Research Laboratories). 9th ed. Compiled by MYRON J. RAND. The National Research Council, National Academy of Sciences, Washington, D. C., 1950. 444 pp. 17.5 x 25 cm. Price $5.

This useful book contains information on the research laboratories of 2,845 industrial, commercial, and institutional organizations. The information presented includes the names of the president and research executives, the location of the laboratories, the numbers and kinds of research workers and other personnel of each laboratory, the fields of research activity, a description of laboratory facilities, and information as to whether or not consulting services are offered. Material for this ninth edition was gathered between January and June, 1950. It was obtained by sending explanatory letters and information forms to about 7,000 companies, including the 2,443 listed in the eighth edition. Decisions on what constitutes "research" and who is a "scientist" were left to the organizations queried. As a rule, these terms were broadly interpreted because of the definition in one of the explanatory letters sent out by the National Research Council stating that "the term 'research' for purposes of the directory, is considered to include industrial development work in processes, equipment, and production, as well as fundamental and applied research; it does not apply to laboratories concerned only with production control or commercial testing." Thus the information presented in the ninth edition of *Industrial Research Laboratories of the United States* for each laboratory is based almost entirely upon information supplied by that laboratory.

The directory contains two appendixes, one giving the same information for government laboratories which cooperate with industry, and the other listings for universities and colleges which offer research services to industry, including the kinds of facilities available. The book also contains a geographical index and a complete subject index of research activities. The laboratories listed are arranged alphabetically, which is an ideal arrangement for a directory of this type. The book cannot be recommended too highly to anyone who has occasion to communicate with officials of research laboratories or requires information concerning research laboratory facilities in the United States.

Histamine Antagonists. By FREDERICK LEONARD and CHARLES P. HUTTRER. National Research Council, Washington, D. C., 1950. 122 pp. 17.5 x 25 cm. Price $1.50.

In this book the pharmacological properties, methods of *in vitro* and *in vivo* evaluation, methods of synthesis, structure-activity relationships, mechanism of action, and clinical application of histamine antagonists are discussed. More than 1,000 compounds which have been tested for potential antihistaminic activity are listed in 48 classified tables including structures, activities, and literature references. Emphasis is placed on the analysis of structure, activity, correlations within the different classes of compounds, and on generalizations which can be deduced. The review contains 224 references.

Official Methods of Analysis of the Association of Official Agricultural Chemists. 7th ed. HENRY A. LEPPER, Chairman, Editorial Board. Published by the Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D. C., 1950. xv + 910 pp. 16 x 24 cm. Price: Domestic, $10; Foreign, $10.50.

In this seventh edition the A. O. A. C. presents its methods, including all revisions which have been adopted since the publication of the sixth edition in 1945. [THIS JOURNAL, **35**, 224(1946)]. All changes in methods adopted up to, and including, those made at the annual meeting of October, 1949, are given.

It will be noted that the designation "tentative" has been deleted from the title. This deletion reflects the action of the Association in sponsoring official and first action methods and procedures. Many new methods have been added to the seventh edition but the number of pages has been slightly reduced. This was accomplished by the introduction of conventional abbreviations for customary laboratory operations.

The chapters of the book are divided into six general groups, in keeping with the materials with which they individually deal. These six general groups are: (*a*) soils and materials related thereto; (*b*) miscellaneous materials not foods or drugs; (*c*) foods; (*d*) drugs and cosmetics; (*e*) general methods; and (*f*) reference tables. This classification has resulted in a more logical sequence of subjects than was obtained in previous editions of *Official Methods of Analysis*. It illustrates, also, the diverse coverage achieved by the methods presented, which are designed for the analysis and examination not only of products of, or essential to, agriculture, but also applicable to other commodities not too closely related to agriculture.

Changes in methods will undoubtedly be made in the interim between the appearance of the seventh edition and the publication of the eighth edition. Provision is made to enable each purchaser of the book to obtain, at cost, reprints of the reports on changes in the methods adopted in the annual meetings of 1950–1953, inclusive.

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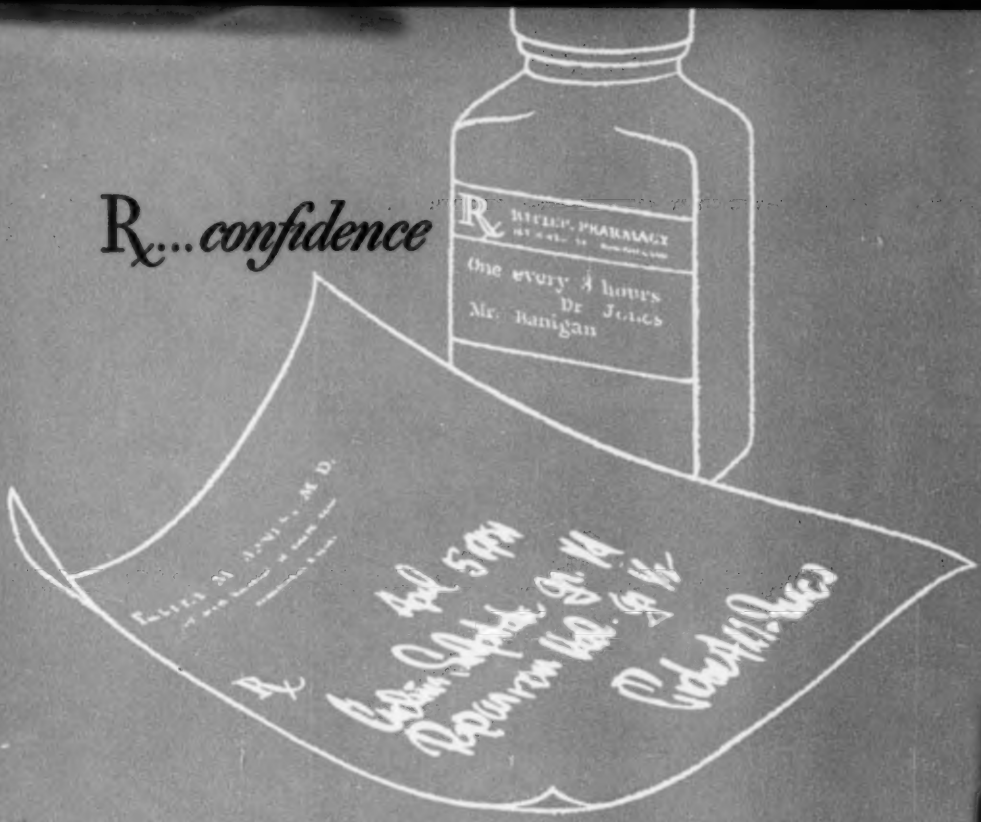

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